INCREASED SENSITIVITY IN EXTRACTION-PHOTOMETRIC AND FLAME PHOTOMETRIC DETERMINATIONS

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INCREASED SENSITIVITY IN EXTRACTION-PHOTOMETRIC

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SUMMARY

Several approaches are explored for increasing the sensitivity of two types of analytical methods: extraction-photometric determination and flame photometric determination. The sensitivity of photometric determinations is increased by increasing the magnitude of the parameters: effective path length, b, and effective concentration, c, of Lambert-Beer's law, A = abc. This increase in b or c has provided increased sensitivity, either with methods that increase the parameters singly, or with methods that increase both. It is felt that the techniques developed can be used successfully in practical analysis when low concentrations or small samples or both are encountered. The sensitivity of flame photometric determination is increased by the use of a standard addition technique with partial sample consumption.

The previously described technique of solid extraction is extended to a wider variety of systems. The effectiveness of this method in achieving both enrichment and phase separation is demonstrated with applications to both micro extraction and enrichment extraction. This preconcentration step yields a solvent bead containing the species of interest which may then be subjected to a wide variety of instrumental finishes. The most common instrumental finish to extraction, spectrophotometry, is applied both with a modified Spectronic 20 and with a long path photometer capable of incorporating microcells up to 50 cm in length. The total volume of liquid needed to fill the cells of either instrument is less than 5 ml.

Photometric titrations are performed using the long path photometer as a phototitrator. With the large increase in path length made available by this instrument, titrations can be performed at concentration levels much lower than previously reported. Procedures are given for reduction-oxidation titrations and for chelometric titrations, in most cases using well established procedures with appropriate volume modifications.

A method for standard addition is presented which is useful when operating at or near the limit of detection. The technique consists of withdrawing a volume 2S of the original volume V, to obtain a scale reading R. A volume S containing Q ml of standard of concentration W is added to the remainder of the sample solution, and the process is repeated several times. The set of R values corresponding to the number of steps, n, is plotted, and the zero intercept with the x axis, P, is obtained. This negative value is used to calculate the original concentration C of the sought-for species by the equation

$$C = \frac{P*Q*W}{P*S-V+S}$$

This graph yields a straight line curve only when the volume withdrawn is twice that replaced. A computer program is given to obtain the best fitting line via a least squares approximation, to perform the calculation of C, and to print out the results.

This method avoids aliquotation of the original sample and the consequent reduction in values for the scale readings which are obtained in the usual standard addition technique. All the measurements are performed

on the original sample, or sample added standard, thus affording greater sensitivity.

CHAPTER I

INTRODUCTORY REMARKS

The work presented in this dissertation is in three different areas: solvent extraction, long path photometry, and standard addition in flame photometry. It may seem that the areas of this study are not interrelated. However, this is not the case; the various methods and techniques described are connected, and strongly so, by the common goal of attacking some of the problems inherent in low-level determinations.

The first section of the work deals with the development and extension of a technique called "solid extraction". This approach aims at the improvement of solvent extraction as a separation technique with particular emphasis to concurrently enhancing enrichment. Description is given of the advantages unique to the use of solvents which solidify at or near room temperature resulting in a solvent bead containing the species of interest. A wide variety of instrumental finishes may then be applied to the solvent bead.

The second section deals with the most commonly used instrumental finish, that is, spectrophotometry. Here the problem arose of fully realizing the considerable enrichment achieved by extraction into a very small bead. Most commercially available instruments require an amount of sample liquid that would necessitate diluting the solution of the beads to a point where the enrichment is severely diminished or even lost. To

remedy this difficulty, a long path spectrophotometer had been developed by Barnes (1). Modifications were made on the original instrument to permit a wider variety of samples to be used with both spectrophotometric determinations and photometric titrations.

The third section describes another available final processing of the solvent beads, namely flame photometry. A technique was developed which avoids splitting the sample into several aliquots, which after addition of standards are diluted to known volume. Instead, several measurements are performed on the original solution with alternating portion withdrawal and standard addition. A mathematical treatment of the problem is given which prescribes the relative amounts of standard added and portion withdrawn so that a linear relationship results between measured quantity and concentration. This greatly facilitates processing of the data.

CHAPTER II

SOME GENERAL CONSIDERATIONS IN LOW-LEVEL DETERMINATIONS

The need for accurate, precise, and rapid methods for low-level determinations is obvious at a time when great emphasis is placed on the awareness and control of traces both in the environment and in consumer products. The problems of identifying and quantitating small amounts of one or more substances in the presence of large amounts of others are by their very nature a challenge to the skill and training of the analyst. Often such problems are interdisciplinary in the truest and finest sense of the word. The legal, economic, and social implications of low-level determinations often demand that specialists in these areas take an active part in defining and solving problems of this sort. Such agencies as the FDA were created among other reasons out of the necessity of defining acceptable levels of toxic materials in food and drugs, as well as checking the compliance with these defined limits. Literally millions of lives depend on the accuracy of the methods used by this and similar agencies.

Applications involving low-level determinations are typically characterized by their diversity. This diversity is not only in the level of complexity of the problem but also in the absolute amounts (or concentration levels) of substances to be determined. Sample size often poses an additional restriction. Samples of river water, snow, ore, or insecticide contaminated food, as well as a host of other examples, are not usually limited in any practical way as to the size of sample available

for analysis. There are, however, many situations which arise where the sample size is inherently very limited. Clinical chemistry is by its very nature concerned with samples of restricted size--a few tenths of a milliliter of serum or a milligram portion of tissue may be all that is available. Similarly, the analysis and characterization of extraterrestrial material is of necessity carried out on samples of restricted size.

The levels of concentration at which a quantitative answer is desired have changed in many applications from the part per million to the part per billion and even part per trillion. As concentration decreases linearly, the difficulties of analysis increase exponentially at every point. When operating at the range of sensitivity required to detect and determine at such levels, several factors must be considered in light of these extreme conditions: the size and reproducibility of the blank, the necessity for standard or reference materials, and the number of manipulations.

The Blank

It is important to realize the twofold meaning of the word "blank". First, the liquid (or solid) preparation which is prepared in exactly the same way as the sample but without its addition is sometimes referred to as the blank. Secondly, the numerical value which results from a measurement on this preparation is also called the blank, or better the blank value.

This value reflects the overall variation stemming from two different sources that contribute separately and independently: the variation in the liquid or solid preparation, which will hereafter be referred to as the "chemical variation," and the variations in the measuring instrument

itself, which will be referred to as the "instrumental variation." The chemical variation arises largely from impurities in the reagents and glassware used and is usually more varied and of higher value. The instrumental variation is due to sixty-cycle noise, shot noise, drift phenomena, variations in the performance of electronic components with temperature, and a host of other sources. With the advent of highly stable, low noise instrumentation, the variation due strictly to the instrument can be made very small. The limiting factor then becomes the chemical variation.

An example of the total variance of a measured value depending on both factors is seen in studies by Kaden (2) related to spectrophotometry. The scatter in blank values was shown to be independent of the path length of a cell from 5 to 50 mm. These results point to the limiting factor in the blank value variation being the chemical variation. With the increase in path length, the total of the two variations approaches a limit, that of a relatively large chemical variation and a smaller instrumental variation which reaches a limiting value. Beyond this point, the overriding factor is the chemical variation.

Consideration of the causes of the chemical variation is of paramount importance in trace analysis, since these causes are less predictable and more likely to create variation than the instrumental sources in a given set of measurements under the same prevailing conditions. Arriving at a low, reproducible blank value is fundamental to meaningful results. There is no lack of contributions to the chemical literature on this subject. One very significant development in reducing the size of the blank value has been the recent production of extremely high purity reagents,

usually with certified analyses of the amount of traces. These "ultrapure" reagents have been instrumental in the successful analysis of extraterrestrial material (3) and a myriad of other applications where the limiting factor was the purity of reagents used with respect to the substance being determined. Much still remains to be improved, particularly in storing such products in such a way as to avoid contamination. Even if the perfect noncontaminating container is fabricated, the problem of the cleanliness of the laboratory would still prevail. The typical uncontrolled laboratory can contain up to 200 μg of dust per liter of air. Representative values for the chemical content of such particulate contamination are: Ca-10%; Cu-5%; Fe-3%; Si-1.5%; Ni-1%; K-1%; Al-0.5%; Mn-0.5%; and traces of other elements (4). Special hoods or clean rooms under laminar flow of efficiently filtered air under positive pressure are effective in reducing chemical contamination during processing steps in the laboratory or factory (5). In addition to these techniques, the use of nitrogen purged glove boxes is sometimes warranted in very careful work. Ideally, all metals would also be eliminated from the construction of such "clean rooms".

A direct relationship exists between the magnitude and variability of the blank value and the limit of detection. The following equations are given:

$$1) \quad \underline{x} = \overline{x} + ks$$

$$2) \quad \underline{c} = f(\underline{x})$$

$$3) \quad s_{b(tot)} = \sqrt{s^2_{(chem)} + s^2_{(instrum)}}$$

where $\underline{x}$ is the value of the measured quantity at the limit of detection, $\overline{x}$ is the average value of the measured quantities, "b" refers to the blank, "k" is some integer, s is the standard deviation, and $\underline{c}$ is the concentration at the limit of detection (6). The value of k is sometimes taken to be 3 (7), although a number of workers have used the value 2. The meaning of equations 1) and 2) is best shown by illustration. Figure 1 shows an analytical function which follows equation 2). The slope of this curve is usually called the slope sensitivity of the method, that is, how much change in the measured quantity results from a given change in c. This slope sensitivity is independent of the limit of detection, $\underline{x}$. Given a value for the average of the blank and the standard deviation, it can be seen that the f(x) curve passes through $\underline{x}$, and below this value the curve has no value for chemical analysis.

A blank of the type discussed still does not adequately compensate for the influence exercised by material other than the one of interest. Such influence is termed a matrix effect. For example, in an X-ray fluorescence determination of a trace constituent in an alloy, the presence of other elements in the sample may have a large effect on the signal from the species of interest--either positive (enhancing) or negative. In cases such as these, sufficient accuracy is obtained only by comparison to a standard material of similar composition and known content of the species of interest.

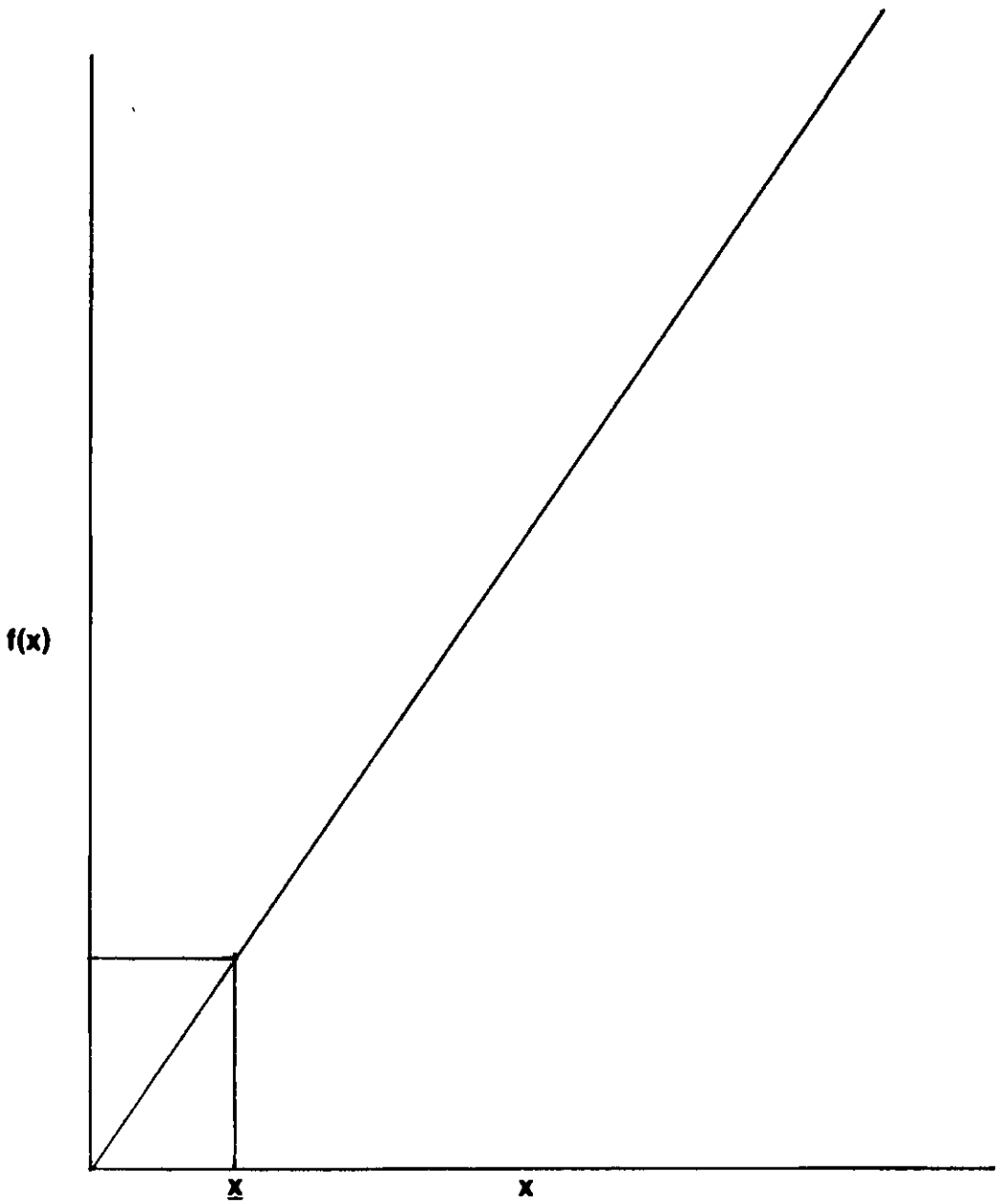

Figure 1

Function Following Equation 2

Standards

The simplest case of the application of a standard for low-level determination is the volumetric dilution of a stock solution of known content of the species to be determined to appropriate values to use in the construction of a calibration curve or in the calibration of an instrument. Although this approach is appealing in its simplicity, it is subject to several difficulties. First, it is applicable only to such systems where matrix effects are absent. Studies of trace substances in air and water samples sometimes meet this requirement, and a great use of such standards has been made for this type of determination. However, the dilute solutions used are adversely affected by several factors, including: adsorption of the substance to be determined on vessel walls; reduction or oxidation to an undesired state; leaching of material from the container; desorption of previously contained material; or chemical instability (decomposition).

Another approach is to prepare a blank with the matrix material added. While this is useful for a number of analytical methods on the macro scale, it is not generally applicable to trace analysis, primarily because of the purity requirements of the materials used. Many matrices are so complex that they are prohibitively difficult to duplicate, such as biological matrices or some mineral matrices.

The most useful standards are usually synthetic standards or analyzed reference materials. Unfortunately for trace analysis, these are very difficult to obtain. The efforts of NBS and others have resulted only recently in standards of both types for trace analysis. An example

of a synthetic standard is high purity glass doped with some 60 metallic oxides at concentrations of 500, 50, 1, and 0.02 ppm (8). Analyzed reference materials include orchard leaves, bovine liver, and albacore tuna with certified content of metals at the part per million level (9). Even if some technological breakthrough were to make possible the generation of a large number of such certified standards, the problems of storage, handling, and stability of at least some of these materials would still exist. Other problems also arise in either type of standard. In the case of synthetic standards the overriding concern is the purity of the constituent materials with respect to the species to be determined. In the specific case mentioned above, much expertise and effort was necessary to fabricate an "ultrapure" glass needed as the starting material for the desired standards. Although it would seem easier to avoid the stringent purity requirements of constructing a synthetic standard by using available materials and simply analyzing them, the techniques needed to analyze for the substances of interest are often either unavailable or require synthetic "knowns" to calibrate the instrument involved.

One way to overcome these difficulties is to employ a standard addition method, one of the most widely employed techniques to overcome matrix effects. In this particular case the "standard" is a solution (or solid material) with known content of the species to be determined. This material is added in increments to the original sample or aliquots of the sample, measuring some parameter related to concentration after each incremental addition. This added material is of relatively high concentration and needs only to be free of the matrix elements whose effect is

being circumvented. It is assumed in such a technique that the sought for substance added will be affected in exactly the same way by the matrix as that already present. A graphical analysis or calculation of the results of measurements on samples so treated gives the desired answer. A method of standard addition which is particularly applicable in cases where sample size is restricted is given in Chapter IX.

Number of Manipulations

In any analytical procedure, consideration must be given to the number and type of manipulations involved. In general it may be stated that the fewer the number of manipulations, the smaller the error; all other factors being equal. Certain operations inherently generate errors and must either be circumvented or their influence minimized when used in trace analysis. A striking example is that of evaporation of a sample. Any traces of volatile materials will of course be lost (and usually to a non-reproducible degree). Of even greater concern, materials which under usual conditions are non-volatile may exhibit unusual volatility. The danger of contamination during evaporation is great, and appropriate measures must be taken to minimize this potential difficulty. Such considerations have lead to the widespread use of techniques which require the minimum of chemical or physical pretreatment before measurement is made. With proper attention to potential sources of difficulty, such pretreatment is valuable or even necessary for optimum results from such "trace" methods as activation analysis. Whatever the method of analysis, it is always important to minimize the amount of contamination and chemical or physical losses before measurement.

Conclusion

In an effort to resolve some or all of the above problems, various approaches were taken in the work to be described. These efforts are along the lines of enrichment, separation, long path length spectroscopy, and standard addition.

The time honored technique of extraction for enrichment and separation has been expanded and improved with the use of so called "solid solvents". An additional application to small samples with low concentrations of the species to be determined is also described.

Very closely related to enrichment and separation is the use of a spectrophotometric finish to make maximum use of enriched samples. A long path spectrophotometer which requires small volumes to fill the cells is used to obtain large absorbance values with minimum volume requirements. Photometric titrations were also performed using this instrument.

Flame emission and atomic absorption spectroscopy, both of which consume the sample, have been applied with a technique of standard addition with partial sample consumption. This method conserves material, and is particularly applicable when working at concentrations near the limit of detection because it avoids aliquotation of the sample.

CHAPTER III

EQUIPMENT AND CHEMICALS

Chemicals

Distilled water was obtained from a Barnstead still equipped with a Ventguard filter. The water was then passed through a mixed bed deionizer. Water prepared in this way was used throughout except for the microtitration of permanganate, where distilled water was used. All common acid, base, and buffer solutions were prepared from reagent grade chemicals. Metal salt solutions were prepared from J. T. Baker "Analyzed Reagent" grade salts, except for some of the copper solutions for microtitration, which were prepared from "Fisher Certified" 1000 part per million copper solution.

Organic chemicals varied in grade from technical to reagent grade, depending on their use. All compounds used in the solid extraction technique showed no indication of trace metal contaminants when tested with PAN.

Glassware

Class A glassware was used throughout without additional calibration. Micro glassware was used extensively in the micro extraction work and with the photometric titrations. Common laboratory glassware such as beakers and flasks were used as needed.

Microburets

A Gilmont microburet of a Coleman Microtitrator Model 6-281 was

employed for the photometric titrations described in Chapter V.

pH Meter

All required pH measurements were made with a Corning Model 7 pH meter. This instrument was calibrated with either a 0.05 F KHP solution--pH 4.01, or a Leeds and Northrup pH 6.86 buffer.

Spectrophotometers

The spectrophotometric measurements described in Chapter III were carried out either on a modified Spectronic 20 modified to permit use of 1.5-ml samples (10) or with a long path length spectrophotometer previously described (11). Some of the photometric titrations were carried out using a 20-cm "all-glass" cell also previously described.

The absorbance curves were obtained with a Bausch and Lomb Spectronic 505 Spectrophotometer.

The atomic absorption measurements were obtained on either a Jarrell Ash 82-270 Atomsorb Atomic Absorption Spectrophotometer or a Perkin-Elmer Model 403.

A Coleman Model 21 flame emission spectrophotometer was used for measurements in Chapter IV.

CHAPTER IV

SOLID AND HOMOGENEOUS EXTRACTION

Solvent extraction has been extensively employed in analytical chemistry, primarily to effect separations. When enrichment of the species of interest or micro extraction techniques are needed, the recently developed methods of solid and homogeneous extraction are useful The basic philosophy of these methods and an historic view of their deduction has been given by Barnes (12). Here one can restrict oneself to the mere descriptive definition of the terms as follows. "Solid extraction" refers to a technique in which the organic phase, which is liquid during the actual extraction process, is solidified to facilitate its withdrawal from the aqueous phase. This approach is readily realized by employing as extracting solvent a suitable compound that is solid at or near room temperature. "Homogeneous extraction" refers to the use of an organic compound that is highly soluble in water at elevated temperatures and that separates on cooling to room temperature. When a compound is used that is solid at room temperature with the same solubility characteristics described, the cooling initiates both separation and solidification and the process is then described as "solid homogeneous extraction."

Extension and expansion of the preliminary work described in (12) was undertaken in order to achieve a more complete understanding of the phenomena involved as well as to expand the possibilities of useful applications of such techniques. The investigation was in two primary areas:

the evaluation of desirable characteristics of "solid solvents", and the use of these characteristics to select further solvents and evaluate their usefulness in extractions.

Solvent Characteristics

Preliminary observations of the behavior of solid events has led to the conclusion that several parameters are of interest for the selection and evaluation of substances for use. These are: melting point; tendency toward supercooling; viscosity of the molten solvent and its interfacial tension with the aqueous phase; possible color enhancement by the solvent; solubility; and the availability and purity of the material.

With the melting point as the sole criterion for solvent selection, the upper limit is obviously given by the boiling point of the aqueous phase. As a first approximation, about 10 to 15 degrees below this point would be a reasonable limit. This selection is influenced by practical considerations such as comfortable handling of extraction vessels or the tendency of some systems to bump or foam excessively at elevated temperature. The lower limit when considering only ease of solidification would be about ten or so degrees above room temperature. However, the selection of the lower limit must take into account the tendency of the compount to supercool. While a substance melting at 35 degrees is of course a solid at room temperature, it may require cooling with an ice bath to initiate solidification. Consequently the lower limit is more practically selected with supercooling in mind. Room temperature should provide a sufficiently low temperature to take care of the supercooling range. These considerations were verified in practice with the first substances investigated

for use as solid solvents, namely, naphthalene (m.p. 80°C), which was used by Fujinaga and co-workers (13), and its 2-methyl and 1-nitro derivatives (m.p. 38° and 50°C, respectively). Of these, the 1-nitro derivative was the most suitable from the standpoint of melting point. The 2-methyl derivative requires an ice bath for solidification, and the naphthalene needs special handling of the heated extraction vessel to avoid burns. Additionally, naphthalene sublimes, making it unsuitable for weight aliquotation.

While supercooling is a nuisance with simple solid extraction, it is an essential feature with solid homogeneous extraction. An extraction leading to a solid organic phase can only be homogeneously performed if supercooling occurs and the solvent persists in the liquid phase for some time. If solidification is immediate, then no homogeneous extraction takes place--instead the solvent simply undergoes a sort of recrystallization. This by its very nature leads to purification and thus to the expulsion of any extracted species. The nonexistence of supercooling is the explanation for the failure of the caffeine-water system to function in homogeneous extraction, even though solubility parameters are ideal for that purpose. The melting point of caffeine is too high to allow supercooling at temperatures less than 100 degrees, so when the upper consolute temperature is understepped the crystals of caffeine immediately fall out of solution without persisting in the liquid phase.

Viscosity and interfacial tension are very important to the successful phase removal of the solidified solvent. For ease of removal, the solvent should ideally coalesce into a single bead. Achievement of this

desired result requires the use of proper technique, since the solvent tends to disperse and cling to the vessel walls. With the use of siliconized glassware and the addition of a drop of dilute surfactant, this difficulty is easily overcome. Sometimes it is necessary to re-warm the system after solvent solidification and melt the solvent again in order to achieve coalescence of the solvent. The use of Teflon or polyethylene ware is sometimes advantageous to minimize adherence of the molten solvent to vessel walls. Since both viscosity and interfacial tension of liquids decrease with increasing temperature, it is usually desirable for satisfactory coalescence to lower the temperature of the system to near room temperature before an attempt is made to gather the droplets.

Another parameter to be considered is that of possible color enhancement of the extracted species by the solvent. The resultant increase in color intensity may permit the use of a particular species which could not previously be used due to low absorptivity. The effective limit of detection may be lowered by such a method, as seen in studies on silicomolybdates (14) and the cobalt-thiocyanate system (15).

The primary consideration in selecting a solvent is that of solubility. Studies by Murata and co-workers (16) have shown that the most important criterion for efficient extraction is the solubility of the species being extracted in the organic phase (saturated with water) compared to the solubility in the aqueous phase (saturated with organic). The ideal solvent would be immiscible with the aqueous phase at all temperatures, with extremely high solubility of the species to be extracted as compared with the solubility of that species with the aqueous phase.

The ideal homogeneous solvent would exhibit negligible solubility with the aqueous phase at the temperature where phase separation is effected with infinite miscibility at elevated temperatures.

Any solvent chosen should be relatively inexpensive, readily available, and either of adequate purity "as received" or amenable to simple purification. The purity requirement can apply to two different areas--both purity with respect to the species being determined and purity with respect to the color of the material if it is to be used with a photometric finish. Most colored impurities can be easily removed with appropriate techniques such as stirring with decolorizing carbon.

In summary, the solvent should have a melting point ranging from about 35° to 85°C, a tendency to supercool--particularly if it has a high melting point, high viscosity and interfacial tension, favorable solubility parameters with respect to the solubility of the species extracted and to the organic and aqueous phase miscibility, and ready availability of a pure or easily purified substance. Additionally, an enhancing effect on color intensity or other measurable quantity of the species to be determined is desirable.

Solvents Investigated for Use

With the above criteria in mind, a number of compounds were investigated for use as solvents. A list of these is given in Table 1, along with their classification as solid or homogeneous solvents, melting points, and solubility data. It is interesting to note that by chance the first solvent selected for evaluation as a homogeneous solvent, piperonal, has so far proved to be the most useful.

Table 1. Compounds Used in Solid Extraction

Compound	m.p.	Type of Extraction	Solubility in Water
benzophenone	48	solid	insoluble
benzophenone, 4-chloro	76	solid	insoluble
biphenyl	70	solid	insoluble
diphenylamine	52	solid	insoluble
2-furanacrolein	54	homogeneous	soluble (hot)
naphthalene	80	solid	insoluble
naphthalene, 2-methyl	72	solid	slightly soluble
naphthalene, 1-nitro	58	solid	insoluble
2-nitroaniline	71	solid	slightly soluble
piperonal	37	homogeneous	slightly soluble to soluble (hot)
thymol	51	solid	insoluble

Examples of applications of some of these substances to enrichment extractions and to micro extractions are given in Table 2. These data were obtained with a Spectronic 20, modified as previously described (10). All extractions were performed with two successive portions of solvent, a technique which has been shown to give an essentially complete extraction in all cases.

Some of the solvents listed in Table 1 merit individual discussion, as their properties are not fully described by the data given. Benzophenone was chosen primarily on the basis of its carbonyl group and its structural similarity to acetone. Since acetone enhances the color of a number of systems, it was hoped that benzophenone would exhibit similar enhancement. This hoped for enhancement did not occur with the systems investigated.

Piperonal was the first solvent investigated as to suitability as a homogeneous solvent. As previously discussed in a dissertation by Barnes (12), when a system other than pure water and piperonal was used, e.g. with the addition of buffer and extracting agent to the aqueous phase, the solubility of piperonal was limited even at elevated temperature. Addition of an auxiliary solvent such as acetone was necessary to achieve complete miscibility. Once this desired miscibility was achieved, the auxiliary solvent was removed by volatilization. An investigation was made of 2-furanacrolein as a homogeneous solvent to compare its behavior with piperonal. The commercially available compound is, unfortunately, of such low purity that it cannot be used as received. This lack of purity, coupled with the difficulty in purifying this compound due to its inherent tendency towards chemical decomposition, makes it unsuitable

Table 2. Enrichment and Micro Extractions with Solid Solvents

metal-extracting reagent, type of extraction, solvent	enrichment factor mg aqueous:mg organic	amount metal, g	absorbances at λ_{max}
Zn- PAN micro extraction 0.2 ml with two 25 mg portions benzophenone	4	0, 0.65, 1.3	0.000, 0.260, 0.500
Cd- PAN micro extraction 0.2 ml with two 25 mg portions benzophenone	4	0, 0.11, 0.22	0.000, 0.018, 0.040
Cu- diphenylcarbazide enrichment extraction 2.0 ml with two 100 mg portions 4 chloro benzophenone	10	0, 0.63, 1.26	0.000, 0.160, 0.330
Cu- Oxine enrichment extraction 0.4 ml with two 25 mg portions benzophenone	8	0, 1.26, 2.52	0.000, 0.055, 0.105
Cu- PAN enrichment extraction 100 ml with two 1 g portions benzophenone	50	0, 63.5 , 127	0.000, 0.305, 0.615

as a homogeneous solvent.

A compound not commercially available that may warrant investigation as a possible homogeneous solvent is o-methyl benzyl alcohol. According to available data on this compound, its solubility in water and melting point (slightly soluble cold-very soluble hot, m.p. 37-39°C) would be ideal. Since synthetic work would be required to prepare this compound for investigation, this compound was not further explored.

The most generally useful solid solvent among the ones listed is 4-chloro benzophenone. The viscosity of its melt and interfacial tension are significantly higher than those of any other compound investigated. The high interfacial tension with water results in unusual ease of coalescence of the solvent after extraction; the high viscosity of the melt prevents dispersion into scattered droplets. The small tendency to supercool makes this solvent easy to solidify near room temperature, with sufficiently low melting point to insure that the compound persists as a liquid long enough for efficient extraction to take place. The only drawback encountered in its use is the tendency of some particularly labile extracting reagents to decompose at the temperature necessary to liquefy the solvent. Dithizone in particular was adversely affected under these conditions.

CHAPTER V

MODIFICATIONS TO THE INSTRUMENT

The prototype of a long path photometer was constructed by Barnes (11) primarily to study the principal aspects and benefits of long path photometry and to illustrate some simple applications to both spectrophotometric determinations and photometric titrations. The main part of the work to be described on long path photometry was undertaken to extend these techniques to a wider variety of systems. When exploratory work was done involving nonaqueous solvents or strongly oxidizing reagents, it was quickly discovered that the original cells did not suffice. Many of the commonly used nonaqueous solvents dissolved the epoxy seal at the end window and photoreceptor. Strongly oxidizing reagents caused their discoloration and decomposition, finally resulting in mechanical failure. Even before that, however, the dissolved, decomposed resin made photometric measurements difficult if not impossible.

Remedy to these problems took two different directions. First, different adhesives were tested on capillary cells of dimensions similar to those of the original one (outside diameter 2 mm, 40 cm length). Second, "all-glass" cells were constructed with a 4 mm inside diameter and of lengths varying from 10 to 40 cm. The adhesive finally used was CIBA 6005, a commercially available product which, after curing, is resistant to the majority of common organic solvents. This was satisfactory for a number of applications, but proved to have the same problem of

decomposition when exposed to strongly oxidizing reagents such as periodate. Consequently, much of the work described was done using "all-glass" cells.

It was frequently noticed that the photoreceptors employed in the original instrument had to be operated near their limit to cope with the reduced amount of light reaching the detector after passing through an additional window. Around that time, phototransistors became commercially available which offer higher sensitivity than the original photoduodiode. There was, however, one major drawback to their use. The previously employed photoduodiodes (Texas Instruments IN 2175 or LS 400) have the light sensitive silicon chip in such a position under an integral lens that only light coming within a rather small solid angle reaches the chip. Consequently, any light outside that angle is of no influence, and consideration of ambient light is minimal. This is not the case with the phototransistors selected for use (Fairchild FPT 110 and 130); these have a broad, flat, light sensitive area and accept light from almost any angle. Thus the higher sensitivity is paid for by the necessity to exclude ambient light. While the insensitivity to ambient light afforded by the photodiodes is a desirable feature, it is not necessary for successful application of the instrument since exclusion of external light is readily possible.

An effect noticed during preliminary work was that of the profound influence exhibited by dust or smoke particles on transmitted light. Consequently, the grating housing and focusing lenses were enclosed in a dustproof seal, painted flat black to avoid light reflection. This arrangement has proven satisfactory. Smoking and excessive moving around

still need to be avoided when doing the most careful work.

Another interesting and unexpected phenomenon was noticed when nonaqueous solvents were used. The proximity of the cell to the hot lamp housing causes a slight warming to occur inside the cell. This produces changes in the index of refraction of the organic solvent and profoundly affects the transmission of light. Several approaches were taken to minimize this effect. The first and simplest approach was to perform measurements before any significant warming could take place. Although this method was satisfactory in some cases, in general it is not sufficient with solvents such as chloroform, whose index of refraction has a large temperature dependence. In such cases, it was necessary to cool the lamp housing or to cap the cell with an appropriate cover. The latter approach proved more successful--a removable jacket of insulating plastic foam, painted flat black on the inside, was fitted over the cell and cell holder.

The electronics of the instrument were virtually unchanged from the previous arrangement. In several cases a McKee Pederson variable voltage power supply was used instead of the one assembled previously for use with the instrument. This was done to take advantage of the greater voltage range available with the McKee Pederson supply. The only other point of significance in the electronics is that the phototransistor, in contrast to the photodiode, requires consideration of polarity when electrical connection is made.

CHAPTER VI

LONG PATH PHOTOMETRY

One of the most frequently used finishes in analysis is photometry. In most cases, 1-cm cells or equivalent tubes are used. According to Lamber-Beer's law, a larger signal would be obtained with an increase in path length. A few higher priced commercial instruments will accept cells longer than 1 cm, but the increase in path length is paid for with a disproportionate increase in volume required to fill the cell. In many cases, achieving this volume necessitates dilution of the solution, and any increase in absorbance is cancelled. Use of cells with long path is therefore of real advantage only when achieved without volume increase. The term "long path photometry" as used here will have the specific connotation of long path with very small volumes. The philosophy of long path photometry has been discussed in a thesis by Barnes (17) as well as several papers by Flaschka and co-workers (11,18).

The benefits of long path and small volume are easily demonstrated with an example. Assuming an absorbance of 0.01 to be a reasonable value for the determination of an absorbing species, and assuming a molar absorptivity of 20,000, a concentration of absorbing species of 2.5×10^{-8} M could be handled with a 20-cm cell. Cells have been constructed in this laboratory which will provide this path length with a total volume of 3 ml, and even less with a 40-cm capillary cell. If an enrichment extraction procedure is at hand that will provide a tenfold increase in

concentration, then 30 ml of a 2.5×10^{-9} M solution could be quantitated. In contrast, a 1-cm cell could handle a concentration of 5.0×10^{-8} M with the same tenfold enrichment extraction.

Much has been said concerning the increase in blank values with an increase in path length (19). The basic argument seems to be that there is no real gain in lowering the limit of detection or increase in slope sensitivity with long path cells. This is ascribed to the larger blank values which would be obtained or more importantly, to the magnification of the scatter in the blank and other values. However, studies by Kaden (20) have shown the scatter in blank values in the system studied by spectrophotometry to be independent of path length from path lengths of 5 to 50 mm. This observation, together with others of a similar nature made in this laboratory, point to a verification of the twofold nature of the total scatter of measured values. As previously discussed, there are two contributions to the variation of a measured value: the "chemical variation" and the "instrumental variation." Kaden's studies seem to indicate that the limit of the value of the scatter of a measurement in spectrophotometric analysis is ultimately the chemical variation, since this contribution is likely to be of higher value and of a less predictable nature than the instrumental variation.

The increase in blank value itself, without consideration of the scatter of the blank value, is thought by some to be a deterrent to the successful use of long path cells. This potential drawback can be avoided easily in practice by the proper selection of conditions under which to make a measurement. The first and most obvious approach is to make the

blank value as small as possible by the exclusive use of reagents which are highly pure with respect to the species being determined. A great diversity of such "ultrapure" reagents has been developed, a large number of which is commercially available. The second method available to reduce the magnitude of the blank value is to exclude or remove as much as possible the interfering substances from reagents. A frequently encountered example of removing interfering substances from reagent solutions is the exhaustive extraction of buffer solutions under the same conditions (pH, ionic strength, excess of extracting reagent) that will be encountered in the actual determination.

Even if every possibility has been exhausted to reduce the blank value, and the irreducible minimum is still relatively high, the difference in the blank value and the solution values, which is magnified by the long path cell, is in many cases equally as useful as the difference between solution values and a nearly zero blank value. Several of the determinations reported were successfully performed under conditions where blank values were high, notably the determination of manganese via oxidation to permanganate. The main concern is that the blank value, however high, is reproducible.

Related to the consideration of the blank value is the reagent background. In many cases encountered in spectrophotometry, a chromogenic reagent is used which exhibits some spectral overlap with that of the species being measured. A classic example of a reagent which has this overlap is dithizone, whose unmetalized spectrum overlaps the majority of metalized form spectra at most wavelengths. When faced with this situation, several approaches are available to eliminate or minimize this

reagent background. The most obvious is to select a wavelength for determination where the absorbance of the free reagent is negligible. This method is deceptively simple, since in long path work the smallest amount of free reagent absorbance is magnified by the increased path length to the point where this absorbance can become of the same magnitude as the absorbance due to the metalized form. Since most determinations are carried out with a considerable excess of free reagent, this may seem to increase the difficulties caused by absorbance of the free reagent. However, as implied before, the main consideration is that the background absorbance be reproducible. Use of a great excess of reagent in most cases is an aid rather than a hindrance in achieving this result. Thus, in addition to the usual reasons for adding excess chromogenic reagent, the added one of arriving at a constant and reproducible reagent blank is important in long path work. With the proper selection of wavelength, this constant background has proven not to be a deterrent to successful results. The second approach to reagent background problems is to remove the excess reagent once the desired reaction has taken place. In a few selected cases, this is a simple and straightforward process, such as in dithizone where excess reagent is removed with a backwash with ammonia.

One of the greatest benefits of long path photometry is the utilization of the many varieties of preconcentration methods to an extent that other methods cannot duplicate. The combination of long path and small volume makes such well established methods as extraction with photometric finish even more powerful. The enrichment possibilities of extraction are taken advantage of to an unprecedented extent. In the particular case

of solid extraction, the greatest benefit of the enrichment extraction would require a cell with a volume less than a milliliter with a length of ten centimeters or more. Such cells have been constructed, and the results of their use in photometric determinations is in progress in this laboratory.

The relatively simple geometry of the cells used in this work is by no means the only available arrangement. The recent advent of low cost, high quality lasers has made possible the use of multiple reflectance cells, which require highly collimated light for successful operation. Cells have been constructed with laser light sources with path lengths of several meters and a modest volume requirement (21). The lack of multiple wavelength lasers to provide several choices of wavelength is the only limit to exploration of this sort at the present time. When such multiple wavelength lasers are available at a reasonably low cost, a whole new area of long path photometry will open up.

CHAPTER VII

PHOTOMETRIC DETERMINATIONS

The theoretical and instrumental aspects of long path photometry have been dealt with in previous portions of this dissertation. This chapter will present a few examples of its application.

Straightforward methods were chosen. This was done since the main point of these investigations was simply to illustrate the inherent advantages of long path photometry and not to improve established procedures or investigate possible interferences.

Determination of Fe with FerroZine

The determination of iron with FerroZine (formula and structure in Figure 2) was introduced by Stookey (22). The magenta complex of iron (II) with FerroZine has a very high molar absorptivity (27,900 at 562 nm) and thus permits the determination of iron at very low amounts (or concentrations) without an enrichment step. If, however, the amounts (or concentrations) are close to the limit of applicability of the method, about 0.1 ppm, enrichment would become necessary--extraction being the simplest approach. However, since the FerroZine complex of iron (II) is not extractible by the usual means, employment of other reagents would be required. Another possible way to obtain larger absorbance values is to employ long path photometry, which allows a considerable downward expansion of the working limits. Stookey's procedure calls for the addition of 1 ml of acid reagent solution to 50.00 ml of sample containing up to

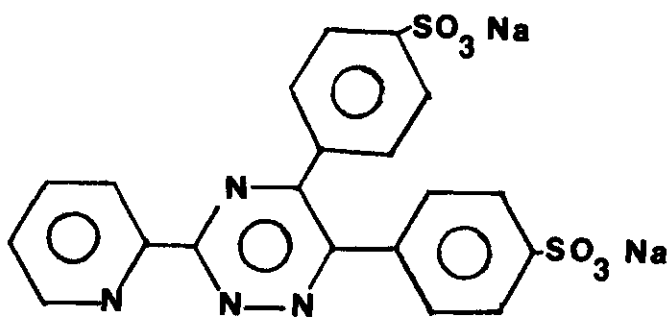

Figure 2

FerroZine-Disodium 3-(2-Pyridyl)-5, 6-Bis(4 Phenyl Sulfonic Acid)-I, 2, 4, -Triazine

4 ppm of iron, boiling for ten minutes, cooling, and adding pH 5.5 buffer. Then it prescribes transferring quantitatively to a 50-ml volumetric flask, diluting to the mark with deionized, distilled water, and measuring the absorbance of the complex formed at 562 nm in a 1-cm cell.

The modified procedure is as follows:

1) Pipet 50.00 ml of sample containing up to 2 μg iron into a beaker.

2) Add 2 ml of pH 4.86 buffer and 0.25 ml of acid reagent.

3) Boil the solution for 5 to 10 minutes. Cool to about room temperature.

4) Transfer the solution quantitatively to a 50-ml volumetric flask, and fill to the mark with deionized, distilled water.

5) Measure the absorbance in a 20-cm cell at 562 nm, and evaluate the reading via the calibration curve obtained in 6).

6) Take known amounts of iron through this procedure, and plot the resulting absorbance values versus amount of iron to obtain a calibration curve.

This procedure varies at three points from the one given in the original paper. First, less of the reagent was used (0.25 ml versus 1.00 ml). This is justified by the absence of interfering metals in the test solutions used. If other heavy metals than iron are present, the additional amount of reagent specified originally insures a sufficient excess of reagent. Secondly, the buffer used was pH 4.86 instead of the prescribed 5.5. However, this pH value is well within the range for optimum complex formation (pH 4-9). Third, and most important, a 20-cm cell was used in place of the standard 1-cm cell.

The calibration curve obtained with known amounts of iron is shown in Figure 3. As can be seen, the values for 4 and 10 ppb are 0.039 and 0.099, respectively. The corresponding values with the 1-cm cell prescribed in the original procedure can be calculated to be 0.0019 and 0.0049. Such low readings would probably be lost in the noise of even a high quality instrument, and would therefore not be usable to obtain a calibration curve. Although the values obtained with the 20-cm cell are not in the optimum range, they are reproducible and can be used.

Fifty-milliliter portions of two "unknowns" containing 0.3 (6 ppb) and 0.4 (8 ppb) micrograms of iron were taken through the procedure to evaluate the method. The results are given in Table 3.

Table 3. Fe Determination with FerroZine

μg Fe/50 ml, taken	μg found	Difference
0.30	0.28	-0.02
0.30	0.31	+0.01
0.30	0.30	0.00
0.40	0.41	+0.01
0.40	0.37	-0.03
0.40	0.41	+0.01

As an interesting sidelight to the above determination, an instrument is currently under construction in this laboratory which is a portable version of the instrument used here (23). This long path spectrophotometer will be used to evaluate the amount of ferrous iron in water

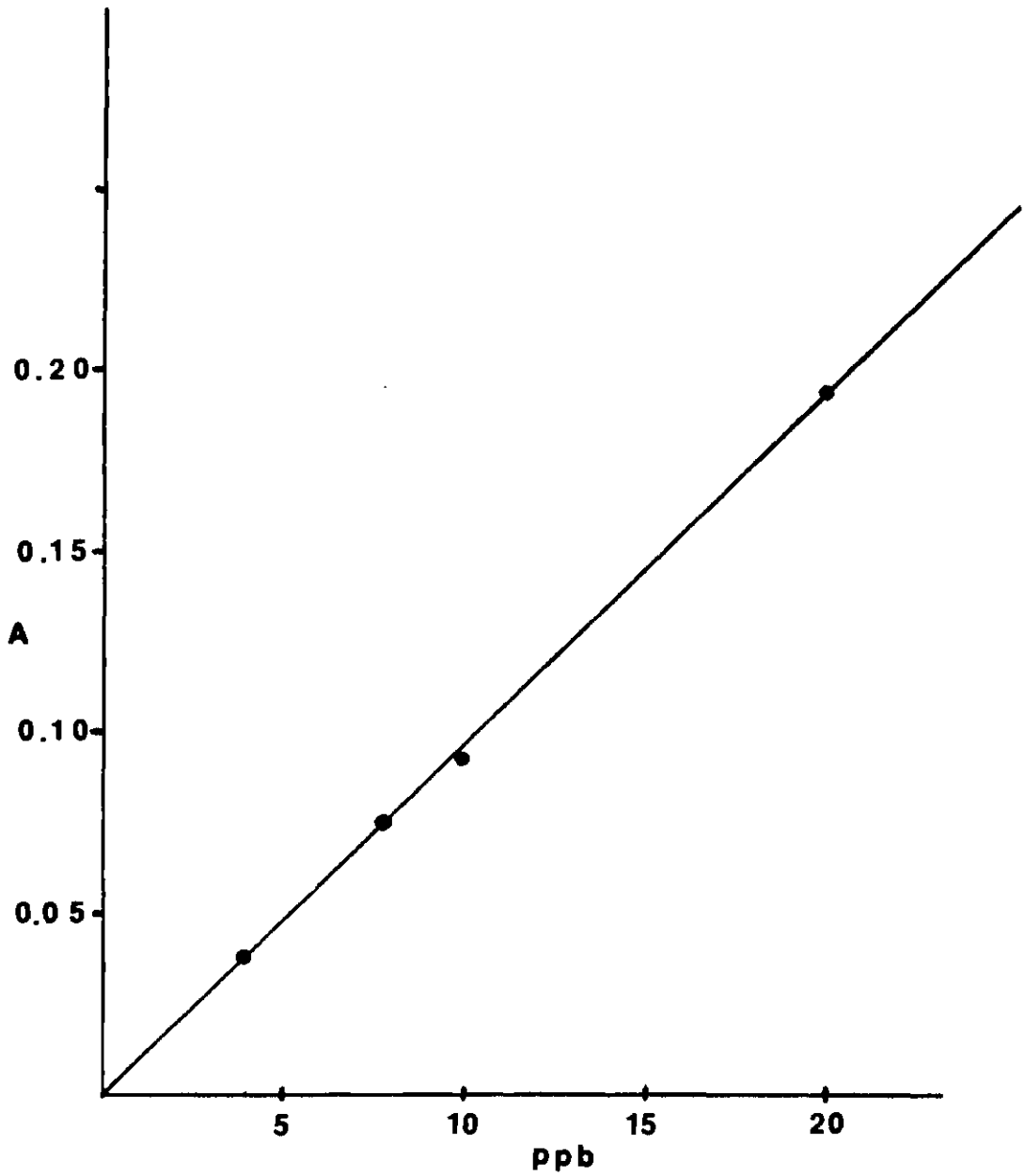

Figure 3

Fe-FerroZine Calibration Curve for Iron Determination

samples, the analysis being performed in situ. An LED (light emitting diode) is used that produces light of a narrow spectral range and can thus replace the combination of exciter lamp and monochromator. Its power requirement is extremely low, and a small battery pack is sufficient to provide power for the LED as well as the phototransistor used as the light detector.

Determination of Cd with PAN

One of the most commonly used chromogenic reagents in extraction/photometric determination is PAN (1-(2-Pyridylazo)-2-naphthol). This compound forms water insoluble, extractable complexes with a wide variety of metals. The stability and high molar absorptivity of these complexes has led to the development of a great number of procedures for the determination of metals in a wide variety of materials.

Since a great amount of work has been done on these complexes in this laboratory, several different determinations were attempted using this compound. The cadmium-PAN system was chosen on the basis of its interest in several previous studies (24). The method followed was based on the one by Berger and Elvers (25). The modified procedure is as follows.

1) Pipet 50.00 ml of sample containing from 0.02 to 0.5 μg of Cd into a flask with a ground glass stopper.

2) Add 2 ml of pH 10 NH_3 - NH_4Cl buffer and 0.1 ml of 10^{-3} F PAN in ethanol.

3) Mix thoroughly and allow to stand for 5-10 minutes.

4) Add 0.1 g of 4-chlorobenzophenone and warm until liquefication of the added solid occurs.

5) Extract the complex into the liquefied solvent by shaking for two or three minutes.

6) Cool the flask under cold water until solvent solidification occurs--the solvent should coalesce into a single bead.

7) Repeat 4) and 5) with a fresh 0.1-g portion of 4-chlorobenzophenone.

8) Transfer the solidified beads to a 5-ml volumetric flask and add a small portion of acetonitrile to dissolve the beads. Make up to the mark with acetonitrile.

9) Measure the absorbance in a 20-cm cell at 565 nm, and evaluate the reading via the calibration curve obtained in 10).

10) Take known amounts of Cd through the procedure and plot absorbance versus amount of Cd to obtain a calibration curve.

This procedure varies from the original at several points. The masking with KCN and formaldehyde is eliminated, since no other metals were present in the samples run. The concentration of PAN was lowered from 10^{-2} F to 10^{-3} F to reduce the reagent background. A solid extraction technique was used instead of the prescribed extraction with chloroform. Most importantly, a 20-cm cell was used in place of the standard 1-cm cell.

The calibration curve from this procedure is shown in Figure 4. The absorbance values for 0.025 μg and 0.062 μg Cd are 0.085 and 0.204, respectively. The corresponding absorbances for a 1-cm cell can be shown by calculation to be 0.002 and 0.005, assuming a final volume of 10 ml of chloroform and a molar absorptivity of 6.1×10^4. These values would be indistinguishable from noise using even the most high quality instrument

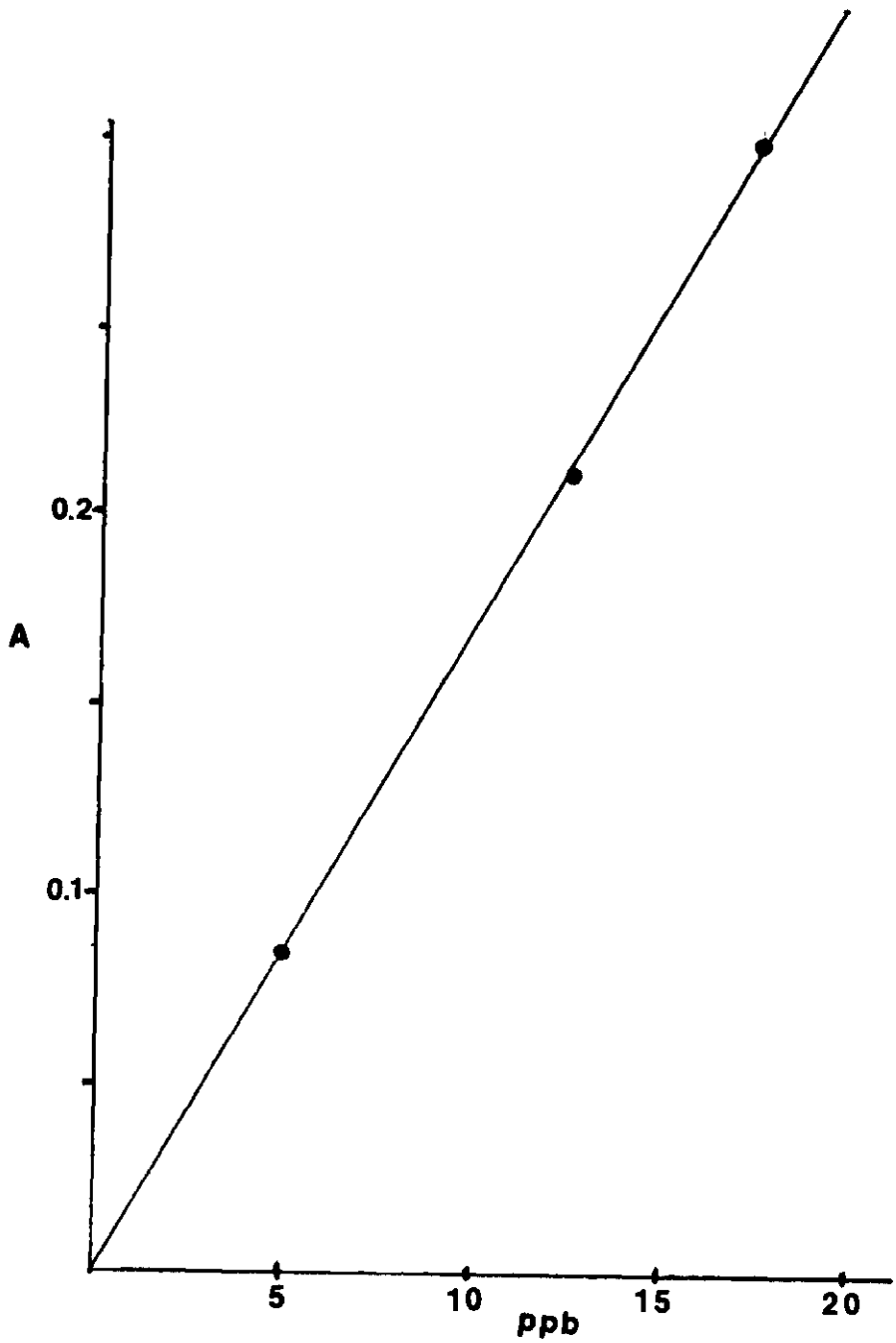

Figure 4

Calibration Curve for Cd-PAN Determination

available. The power of the combination of enrichment extraction and long path finish can be clearly seen.

Fifty-milliliter portions of unknowns containing 0.05 μg (1 ppb) and 0.1 μg (2 ppb) of Cd were taken through the procedure to evaluate the method. The results are given in Table 4.

Table 4. Determination of Cd with PAN

μg Cd/50 ml, taken	μg found	Difference
0.050	0.047	-0.003
0.050	0.051	+0.001
0.050	0.052	+0.002
0.100	0.10	0.000
0.100	0.097	-0.003
0.100	0.112	+0.012

The blank value for the determination with the 20-cm cell was 0.043, which demonstrates the necessity of keeping the reagent background low. Even with 10^{-3} F PAN, the spectral overlap was considerable at 565 nm. Additionally, for satisfactory results in this low-level determination, the buffer used was exhaustively extracted with PAN into chloroform to remove traces of heavy metals. To maintain the chemical cleanliness necessary for such minute amounts of metal, the immediate environment of the instrument was kept as dust free as possible, and the determinations were run after usual laboratory hours to minimize the stirring up of particulate matter.

Determination of Mn in High Purity Sodium Carbonate

The problem of determining trace impurities in high purity materials is a constantly expanding one. One such practical example is the determination of Mn and Fe in high purity carbonate samples which are to be used in the fabrication of glass for optical transmission of signals. This project demands the utmost in purity of the raw materials used, since the slightest trace of transition metals seriously reduces the transmission of light. The Baker Chemical Company, in connection with Bell Laboratories, was contracted to supply carbonates of known high purity to produce such glass. The levels of Fe and Mn were known to be on the order of 100 ppb by neutron activation analysis, and it was desired to evaluate the Mn and Fe content by independent methods.

The samples were received from Baker in polyethylene containers, with the preparation described as follows. In both samples (designated UMO 461 and 32-RD-28), 2.5 g of sodium carbonate was dissolved in 5 F HCl prepared by diffusion, 5 more ml of acid was added, and the resultant was diluted with 18 megohm water to about 25 ml. The sample was then boiled to reduce the volume to about 15 ml, and the pH adjusted to about 3 with NH_3 which was prepared by diffusion. The solution was then diluted to exactly 25 ml with high purity water. An acid blank was prepared exactly as before, with the addition of 1 dr of ULTREX perchloric acid.

Two different and independent methods were chosen to determine the Mn in these samples: A) oxidation with periodate and measurement of the absorbance of the permanganate produced, and B) reaction with PAN and measurement of the red complex formed. For both methods, a long path cell

was used in the final measurement.

Mn by Oxidation to Permanganate

One of the most widely used methods for the determination of Mn is the oxidation to permanganate by any of several oxidizing reagents, and subsequently measuring the purple chromophore produced photometrically. The method is suitable for a wide variety of matrices, is simple and straightforward, and has only chromium as the most commonly encountered interference. The method of Cooper (26) was used with appropriate volume modifications with the high purity carbonate samples.

The modified procedure is as follows.

1) Pipet 2 ml of a sample containing from 0.02 to 0.10 μg Mn into a small beaker.

2) Add 0.5 ml of oxidizing solution (7.5 g potassium periodate, 100 ml of concentrated nitric acid, and 400 ml of 85% phosphoric acid up to 1 liter), and boil for ten minutes.

3) Cool the solution and transfer quantitatively to a 2-ml volumetric flask.

4) Make up to the mark with deionized, distilled water.

5) Measure the absorbance in a 40-cm cell at 560 nm and evaluate the reading using the calibration curve obtained in 6).

6) Take known amounts of Mn through the procedure and plot the resulting absorbance values versus amount of Mn to obtain a calibration curve.

Both this procedure and a modification using the standard addition were used to determine the Mn in carbonate samples. To use the standard addi-

tion method, the following steps were substituted for (6), and two more steps were added.

6a) Add 4 μl of a permanganate solution (0.01 μg Mn/μl in saturated periodate to prevent reduction of MnO_4^-) to the solution in the 40-cm cell.

7a) Read the resulting absorbance.

8a) Repeat steps (6a) and (7a) until several additions have been made and evaluate the results either by calculation or graphically.

The results of determinations done in the two ways described indicated 23.5 ppb by the simple determination and 25.0 ppb by standard addition. These results are in good agreement for this level. The average of these two values, 24.3 ppb, is in agreement with the results obtained by neutron activation analysis by Bell Laboratories (20-22 ppb). The calibration curves for both the simple determination and the standard addition method are shown in Figure 5. The value for the 20 ppb standard was 0.145, which under the same conditions would have been only 0.0036 with a standard 1-cm cell.

Mn with PAN

To check the results obtained by the oxidation method, the samples were also determined by formation of the Mn-PAN complex and photometric measurement of the red species produced. The method of Donaldson (27) was used with appropriate modifications.

The modified procedure is as follows.

1) Pipet 2.00 ml of sample containing from 0.01 to 0.1 μg Mn into a small flask.

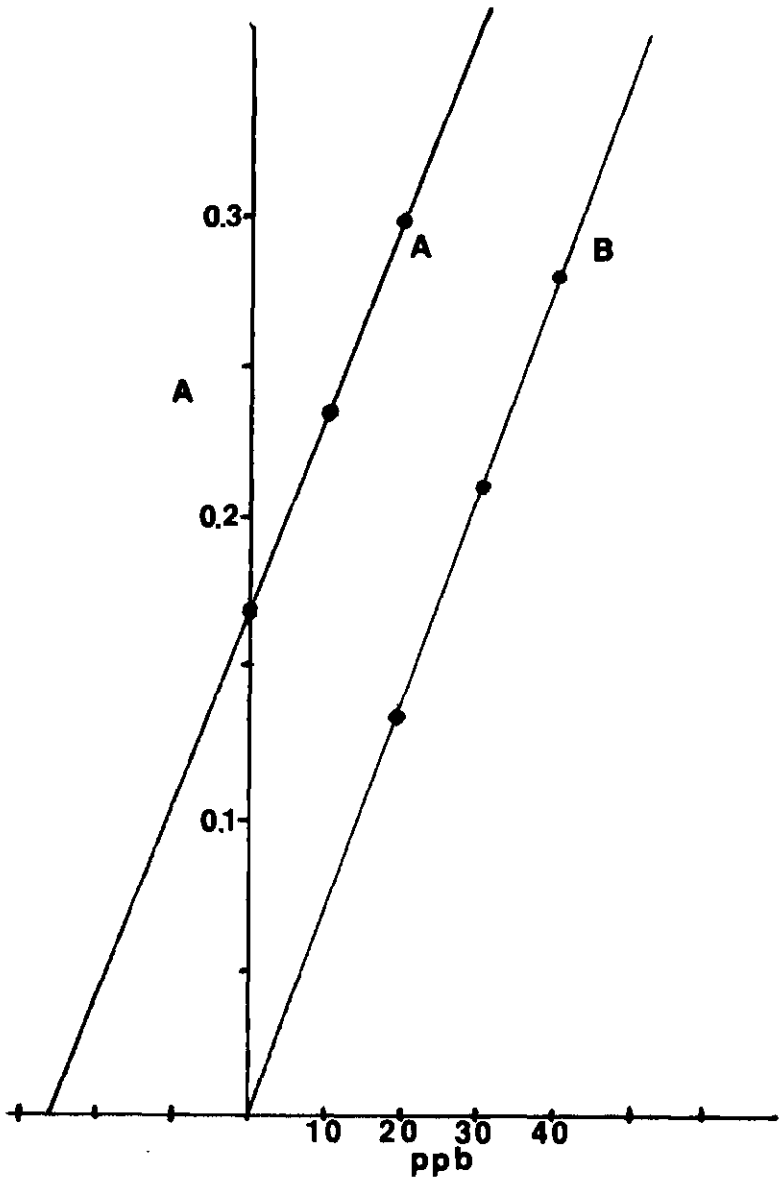

Figure 5

Determination of Mn as Permanganate
A- Standard Addition Method
B- Calibration Curve Method

2) Add 0.1 ml of 10% sodium tartrate solution and 0.4 ml of 20% hydroxylamine hydrochloride solution.

3) Mix thoroughly and add 0.4 ml of pH 10 NH_3 - NH_4Cl buffer (10 g ammonium chloride dissolved in water, 100 ml concentrated aqueous ammonia, and 1.2 g KCN added, and diluted to 200 ml with water).

4) Add 0.05 ml 10^{-2} F PAN in ethanol and allow to stand for 5-10 minutes, shaking from time to time.

5) Extract with two 0.2 g portions of benzophenone, as described in the procedure for Cd with PAN.

6) Place the solvent beads in a small flask on a fast reading (preferably top-loading) balance, tare, and add 3.0 g m-xylene.

7) Dry the resulting solution with a spatula tip of anhydrous sodium sulfate and measure the absorbance in a 40-cm cell at 560 nm, and evaluate the results from the calibration curve obtained in 8).

8) Take known amounts of Mn through the procedure and plot the resulting absorbances versus amount of Mn to obtain a calibration curve.

The value obtained with this method is 19.0 ppb, which is in good agreement with the results from the oxidation method, 24.3 ppb. The calibration curve used is shown in Figure 6. The absorbance value for the 20 ppb standard was 0.505, which under the same conditions would have been only 0.012 with a standard 1-cm cell. The value for the 1-cm cell, though usable, is still much less than that obtained with the long path cell, illustrating the power of the combination of enrichment extraction with a long path finish. With the described procedure, either samples with a much lower Mn content or of smaller size could be handled. This capability to handle smaller samples is particularly important with samples of high cost, such as the high purity material used in these determinations.

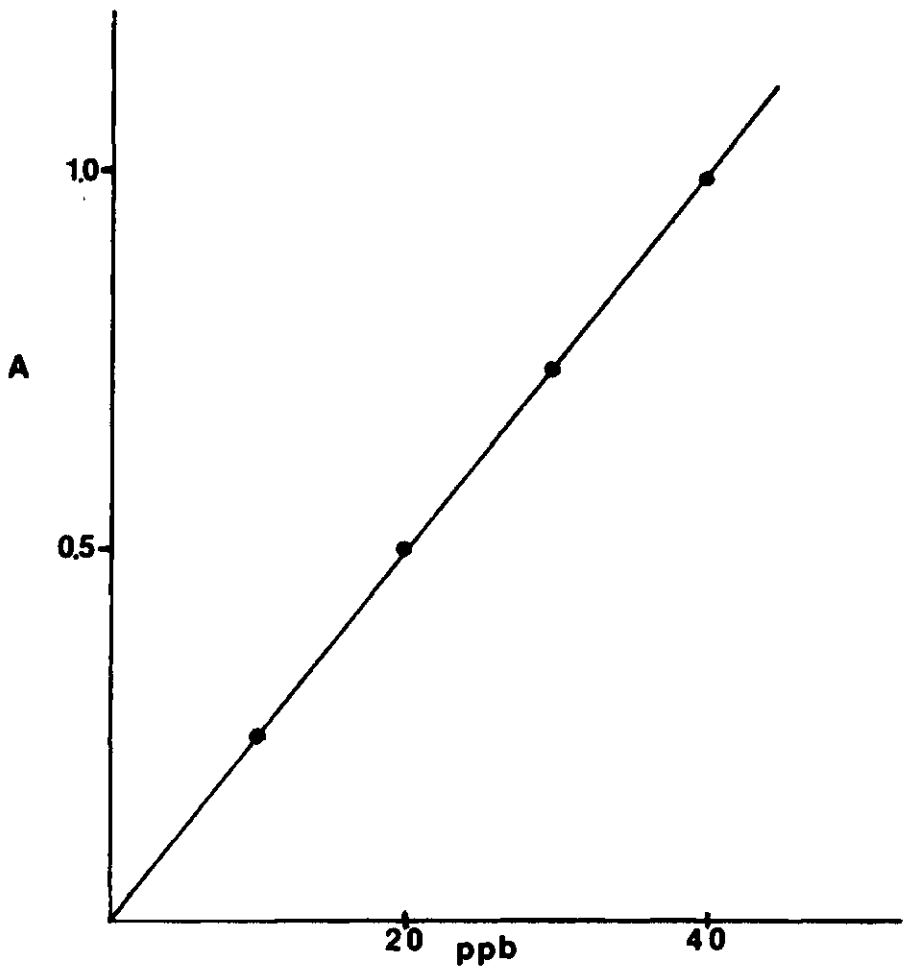

Figure 6
Calibration Curve for Mn-PAN Determination

CHAPTER VIII

PHOTOMETRIC TITRATIONS

The philosophy, advantages, and range of applications of photometric titrations have been extensively documented, even to the point of being included in most introductory courses in analytical chemistry. Consequently, the details of this method will not be presented, and the reader is referred to any of the more prominent texts or monographs which more than adequately handle the subject (28).

Obviously, to successfully perform a photometric titration, there must be a minimum change in absorbance taking place during its course. Up to a certain point, the larger this change, the easier it is to follow the titration. The size of the absorbance change depends on all three parameters in the Beer equation, and an increase in absorbance change may be achieved within certain limits by operating on or with each parameter singly or in conjunction with the others.

Unfavorable conditions may exist in a given titration system due to none of the monitorable species possessing a sufficiently high absorptivity. In this case, it may be necessary to put chemical means to work, most likely complex formation, to convert one or more species into higher absorbing entities. A self indicating titration, the simplest and usually most desirable system, might be achieved in such a manner if the concentration of the monitorable species is or could be made high enough or if the path length could be adjusted long enough. In some cases of this sort,

it may be necessary to abandon the inherent advantages of a self indicating titration system and resort to the addition of an indicator of adequate absorptivity change.

Another possible source of unfavorably small change in absorbance is low concentrations of the monitorable species. Two possibilities present themselves as possible means of circumventing this difficulty. Either the absorptivity of the species may be increased, as previously discussed, or the path length may be increased. Thus, either with low absorptivity or low concentration, the situation may be improved by an increase in path length.

The series of phototitrators developed in this laboratory over the past years took deliberate advantage of the increase in path length to increase absorbance change. There are, of course, limitations to this approach. One cannot increase the path length in full or partial immersion instruments beyond certain limits. Stirring becomes more difficult as the size of the titration vessel increases. The surface area may increase disproportionally with increased vessel size, which fosters more rapid evaporation. This large surface area is detrimental when working with nonaqueous solvents or when working with warm solutions. Most important of all is the discrepancy between the volume needed to accommodate the increase in path length and the volume that is available.

Even when large volumes of solution are available, long path photometry may be helpful. Any suitable vessel may be used for a titration vessel, and the solution circulated through a long path cell with a pump, thus employing the long path photometer strictly as an external measuring device. The more interesting and practically important case is

the one where severe restrictions exist in the available sample size. Here, the long path photometer may be used (with appropriate modification, if necessary) as a phototitrator to accommodate small volumes of solution hampered either by species of low absorptivity at relatively high concentrations or highly absorbing species at relatively low concentrations, or any combination of the two fitting between these extremes. This more important application of the long path photometer is dealt with in the following sections.

Systems Chosen for Titration

In order to demonstrate the applicability of long path photometry to photometric titrations, several different types of systems were chosen. Chelometric titrations were most often chosen. This choice was made not only because such methods are well documented and extensively studied by other means, but also because it is felt that these titrations are more generally useful with long path photometry. Studies were made of turbidimetric titrations as well as reduction-oxidation titrations, but those investigated proved less satisfactory than chelometric titrations.

Turbidimetric Titrations

Employing a precipitation titration with a phototitrator is of course equivalent to performing a turbidimetric titration, since it is irrelevant from the point of light detection whether absorbance changes are caused by "true" light absorption by a chromophore or by deflection or scattering by precipitated particles. It was felt that an investigation of turbidimetric titrations of low concentrations of species to be determined might be profitable, since better titration curves are usually

encountered under these conditions. Both silver and barium were titrated with standard chloride and sulfate solutions, respectively. The results did not show the improvement expected from having low concentrations. Instead, the increased path length served only to multiply the difficulties caused by non-reproducible precipitation conditions and variations due to changes in the vigorousness or amount of stirring. Since the results for these two well-known titrations were so erratic and only rough estimates of concentrations of sought-for species were obtained, this type of titration was not further investigated.

It is possible that future investigation would be profitable with systems of this sort, since with very strict control of stirring, addition of reagents, and other variables, more reproducible results might be obtained. Use of a peristaltic pump might improve the reproducibility of these parameters to a point where good results could be obtained.

Redox Titrations

In order to evaluate the feasibility of a redox titration on small samples with a low concentration of sought-for species, two different iron titrations were attempted. The first titration used the iron (II) ferroin complex as the indicator, and cerium (IV) as the titrant, a well established technique. The red color of the reduced form of ferroin was monitored at 510 nm. The results for this titration were not satisfactory at the part per million iron level, due in part to the slow reaction of the indicator. An additional problem encountered was the size of the indicator blank in relation to the volume of titrant needed to titrate the iron. For these reasons, this approach was not pursued further, and instead the

self indicating titration of iron (II) with permanganate was investigated, with excellent results at the part per million level and below. As previously discussed, a self indicating titration offers the advantage of simplicity. With the relatively high absorptivity of the permanganate titrant, very small amounts of excess titrant can be easily detected, especially with the long path cell. When adequate precautions are taken to exclude oxidizable or reducible impurities from the titration system, there is no detectable indicator blank in the titration performed as described. The only problem encountered was the air oxidation of iron (II), which could be overcome easily by titrating the freshly prepared sample immediately.

The procedure is as follows.

1) Pipet 3.00 ml of sample containing 1 to 20 μg of iron into the reservoir of the long path cell. (The Jones reductor is adequate for prereduction.)

2) Add a few drops of iron-free sulfuric acid to the solution and mix by pulling the solution back and forth in the cell a few times by suction.

3) Set the transmittance to about 90% at 530 nm.

4) Titrate with microliter additions of 0.002 F permanganate, mixing as described, and noting the transmittance after each addition of titrant.

5) Continue with incremental additions until a few readings below 90% T have been obtained. This will provide sufficient points to establish the ascending branch of the titration curve.

6) Convert the transmittance readings into absorbances, plotting the absorbances versus μl of titrant to obtain the titration curve.

7) Draw the best straight line through the two branches of the curve, taking the intersection of these two lines as the end point.

8) Calculate the results in the usual way for the permanganate titration of iron (II).

As a general practice in all the titrations to be described, the titrant prescribed is from 10 to 100 times as concentrated as the solution being titrated, making correction for dilution unnecessary. Several "unknown" samples were taken through this procedure to evaluate the method, and the results are given in Table 5.

Table 5. Self Indicating Titration of Iron with Permanganate

μg Fe/3 ml, taken	μl 10^{-2} N MnO_4^-, calc.	μl found	Difference
5.8	10.5	10.6	+0.1
5.8	10.5	10.5	0.0
5.8	10.5	10.3	-0.2
2.9	5.3	5.2	-0.1
2.9	5.3	5.4	+0.1
2.9	5.3	5.3	0.0
1.4	2.6	2.4	-0.2
1.4	2.6	2.7	+0.1

These results are within the bounds of acceptability with respect to precision and accuracy and indicate that good results can be obtained at this level using the long path instrument without elaborate precautions

or complicated equipment. The trend observed with precision is exactly what would be expected with a decrease in the amount of sought-for species--the less the amount, the poorer the precision, although the accuracy of the average of several determinations at the lowest concentration titrated (0.46 ppm) is quite good.

Chelometric Titrations

Chelometric titrations are more readily applied to photometric treatment for a number of reasons. First of all, there is usually no need to consider an indicator blank in such titrations, since the usual indicator does not consume titrant. Secondly, there is no dependence on oxidation or reduction mechanisms in the cases normally encountered. (This dependence is replaced by the more easily controlled dependence on pH and degree of metalation of the indicator.) A great number of standard procedures have been developed with the specific conditions for titration (pH, masking, choice of indicator, range of applicability) specified. Since such a wealth of information is readily available, and since a wide variety of chelometric titrations has been performed in this laboratory, most of the titrations performed were chelometric titrations.

There is no need to elaborate here on the well established and extensively treated theory of chelometric titrations. Several excellent monographs and books are currently available which cover the various aspects of this method in great detail, not among the least of which is the book by Schwarzenbach and Flaschka (29), as well as the monograph by Ringbom (30). Most undergraduate analytical textbooks also treat this topic in some detail. The purpose of this investigation was to examine several well established systems as to their applicability to photometric

titration with long path photometers. It was felt that these methods could be extended to methods for low level determination.

Studies by Butcher (31) have shown that photometric titrations can be advantageously performed when a self indicating system is created with the addition of an excess of the metal indicator. In most of the examples presented, an excess of the appropriate indicator was added, and the small change in absorbance caused by the removal of the metal from the dye by complexation with the titrant was measured.

A general procedure for chelometric titration with a long path photometer is as follows.

1) Pipet 2.00 ml (with a 40-cm capillary cell) or 3.00 ml (with a 20-cm "all-glass" cell) of solution containing an appropriate amount of metal to be determined into the cell reservoir.

2) Add buffer and indicator, mixing thoroughly by rinsing back and forth through the cell by suction.

3) Set the transmittance to about 10-15% (for upscale titration) or 90% (for downscale titration) at the appropriate wavelength.

4) Add increments of titrant dispensed from a microburet and mix as previously described.

5) Read and record the transmittance of the solution.

6) Repeat 4) and 5) until a few points above 10-15% (upscale titration) or below 90% (downscale titration) have been obtained. This will establish the ascending or descending branch of the titration curve.

7) Convert the transmittances to absorbance values and plot the resulting absorbances versus increments of titrant.

8) Draw the best straight line through both branches of the titration curve and take the end point to be the intersection of these two lines.

9) Calculate the results in the usual way.

An example of a system in which a self indicating method is created by the addition of excess dye is the titration of zinc with EGTA using Zincon as the indicating dye. The spectral curve of free Zincon and the zinc-Zincon complex is shown in Figure 7. To illustrate the possibility of using wavelengths where the difference in absorbance between metalized and free indicator is not maximum, the titration was run at two wavelengths. Both 560 nm and 620 nm, the wavelengths usually selected, were chosen. The actual titration curves obtained are shown in Figure 8. From the curves, it is apparent that only the slope of the descending branch is different for the two wavelengths, due to the difference in absorptivity at the two different wavelengths. The results for the titrations are as shown in Table 6.

Table 6. Titration of Zinc with EDTA with Zincon Indicator

Wavelength	μg Zn, taken	μg Zn, found	Difference
560 nm	13.1	13.3	+0.2
560 nm	13.1	13.2	+0.1
560 nm	13.1	13.1	0.0
620 nm	13.1	13.2	+0.1
620 nm	13.1	13.2	+0.1
620 nm	13.1	13.0	-0.1

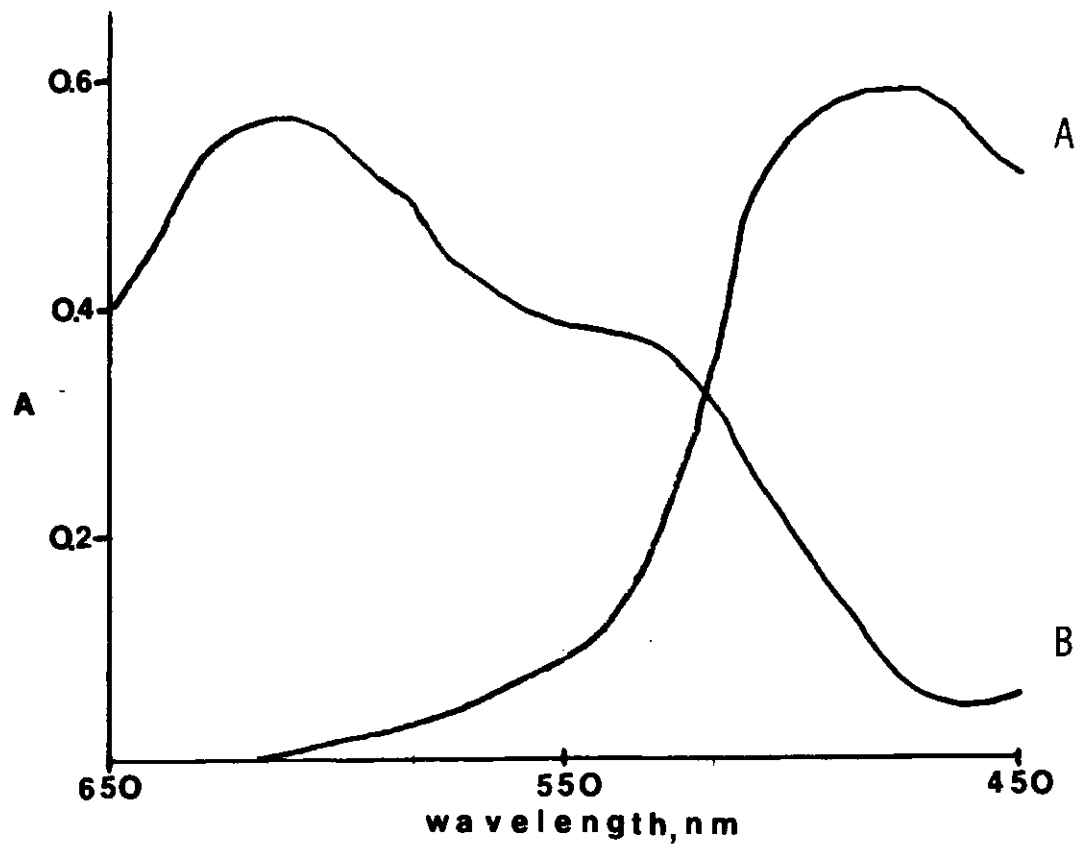

Figure 7
Spectral Curves of Zincon and Its Zinc Complex--Visible Range
A- Free Zincon B- Zinc-Zincon

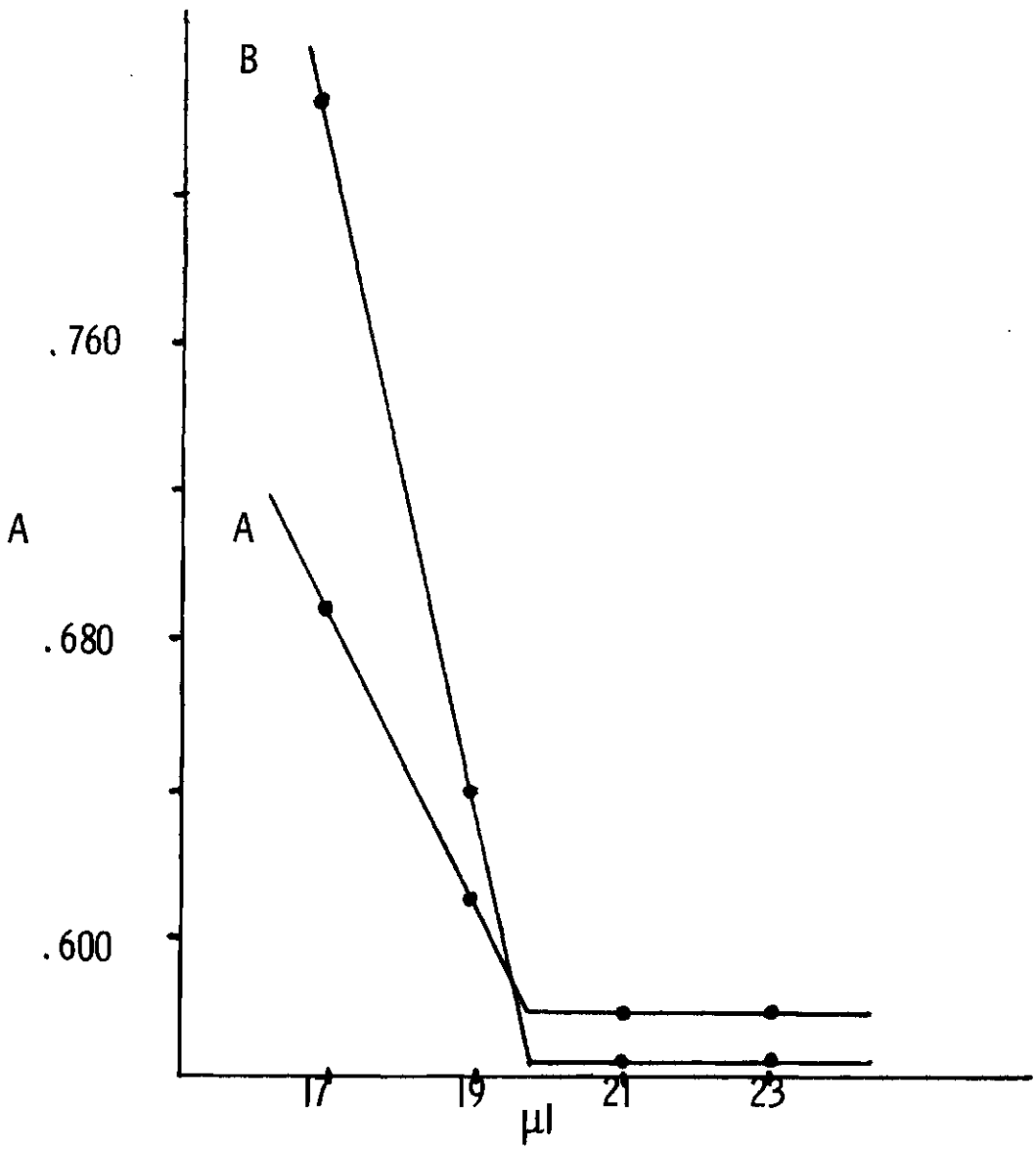

Figure 8

Zinc-Zincon Titration Curves
A-560 nm B-620 nm

These results imply that it is possible to use a system for photometric titration in which the spectral overlap between the free and metalized forms of the indicator is considerable at the wavelength chosen for titration. In fact, in some cases where large absorbances are encountered, it is advantageous to use a wavelength at which the difference in absorbance of free and metalized indicator is not maximum--especially when using long path cells.

Another example is the titration of copper (II) with EDTA using SNAZOXS indicator. The curves for the free and metalized indicators are shown in Figure 9. The titration was followed at 550 nm, where the free indicator absorbs strongly. The amounts of copper titrated are very small, illustrating the potential of the long path photometric method for submicrogram amounts of material to be determined. Known amounts of copper were titrated to evaluate the method. Table 7 gives the results.

Table 7. Titration of Copper with EDTA with SNAZOXS Indicator

μg Cu/2 ml, taken	μg Cu, found	Difference
0.063	0.060	-0.003
0.063	0.063	0.000
0.063	0.062	-0.001
0.063	0.064	+0.001
0.038	0.037	-0.001
0.038	0.038	0.000
0.019	0.019	0.000
0.019	0.020	+0.001

These results are very good for the level of concentration involved (9.5-

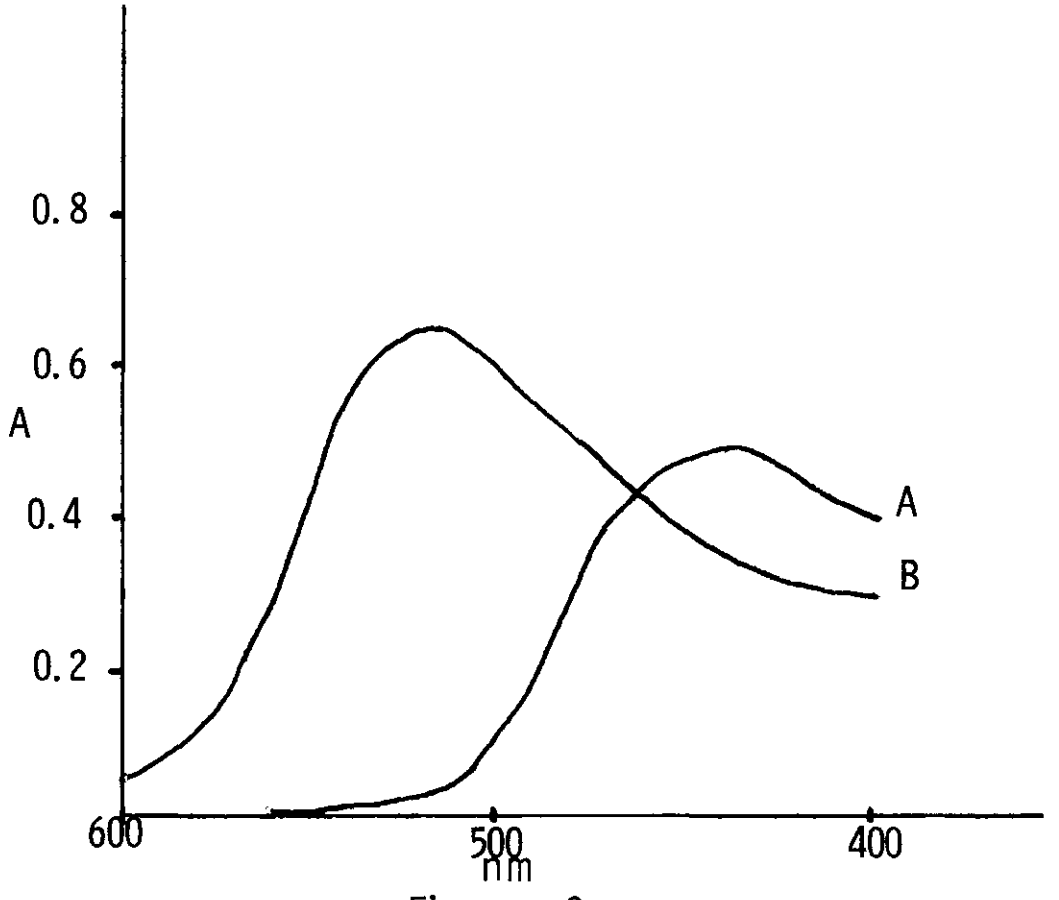

Figure 9

Spectral Curves of SNAZOXS and Its Copper Complex

A- Cu-SNAZOXS B- Free SNAZOXS

31 ppb).

To illustrate the usefulness of the "all-glass" cell, a number of titrations were run with this type of cell. With only a small sacrifice in the amount of liquid required to fill the cell (an extra ml is needed), virtually the same results can be obtained with this type of cell, with the added benefit of the resistance to strongly oxidizing reagents.

Calcium was titrated with EDTA using ARSENAZO III as the indicator with a 20-cm "all-glass" cell with a total volume of 3 ml. This system was chosen to examine the results of a titration where a significant amount of the material to be determined is present in water and reagents, i.e. where the blank is high and a blank titration is needed to obtain accurate results. Table 8 gives the results of the titration of an "unknown" (0.13 ppm Ca).

Table 8. Titration of Calcium with EDTA with ARSENAZO III Indicator

μg Ca/3 ml, taken	μl 5.05×10^{-4} EDTA, calc.	μl found	Difference
0 (blank)	0	4.8	+4.8
0.40	20.0	20.8	+0.8
0.40	20.0	21.1	+1.1
0.40	20.0	20.7	+0.7
0.40	20.0	19.5	-0.5
0.40	20.0	20.0	0.0
0.40	20.0	19.3	-0.7

As an illustration of the contrast between the results of a chelometric method and a redox method with long path phototitration, iron was

run by titration with EDTA using sulfosalicylic acid as indicator, and results of this titration compared with previously described results from the permanganate titration. With the chelometric titration, a concentration level an order of magnitude lower (0.1 ppm versus 1 ppm) was successfully handled with comparable precision and accuracy. In addition, the chelometric titration was faster, and the indicator more stable toward decomposition. In fact, the system with the sulfosalicylic acid indicator gave an indicator complex so stable that the titration could be interrupted for periods as long as an hour without adverse results.

CHAPTER IX

METHOD OF STANDARD ADDITION WITH PARTIAL SAMPLE CONSUMPTION

Introduction

Standard addition is the method of choice under conditions where matrix effects forbid employment of a calibration curve. The technique is straightforward and has been used for decades in such methods of analysis as polarography. The final analytical result in such methods may be obtained either by calculation from a single addition or graphically after several additions. In either case, dilution due to the addition must be taken into account. Multiple additions have been used lately also in photometric determinations under the descriptive name "standard addition titration."

The above discussion has been directed solely towards techniques where no sample is consumed. If, however, sample is used up during the measurement, as for example in flame emission and atomic absorption analysis, simple addition (singly or repeatedly) is no longer possible. In these cases, identical aliquots of sample solution are placed into a series of volumetric flasks and standard solution is added to each flask except one. Then measurements are effected and the scale readings plotted versus amount of standard added. The straight line obtained is extrapolated backwards and the amount of sought-for substance in the sample solution is read at the intercept with the X-axis.

The method works fine and is the standard procedure when small concentrations of sought-for substance or low sensitivity or both result in low-level signals. There is, however, one drawback; the sample solution is divided into several portions and diluted. This is quite undesirable when working near the limits of detection of a determination. It often leads to the painful decision of whether to take many aliquots (causing great dilution) in order to obtain many points for the graph or to resort to a very few aliquots and have the uncertainty of where to draw the best fitting line through a few scattered points.

There is, of course, an alternate possibility, namely, to take aliquots and add standard to them and process the solutions without filling to the mark in a volumetric flask. In such case, however, it would be necessary to add the standard as a solid or as extremely concentrated solution in order to avoid dilution, because in most cases when operating near border conditions (scale expansion, zero suppression, etc.) a simple volume correction cannot be applied. Still, the division of original solution into several aliquots remains. Thus, it seems quite worthwhile to investigate the possibility of designing a method where the original sample solution itself is subjected to additions and measurements.

The principal idea is to take the sample solution, withdraw a certain fraction for measurement, add standard solution, withdraw again a fraction for measurement, add standard, and so forth until an adequate number of points is obtained for the graphing procedure. The feasibility of such an approach is tested on the ground of mathematical considerations in the next section.

Mathematical Treatment

The following symbols will be used.

V initial volume of the sample

C concentration of the sought-for substance in the initial solution

C_n concentration of the sought-for substance after the withdrawal/addition of standard

A volume withdrawn

S volume replaced

T concentration of replacing solution

R meter reading

K proportionality constant

The method of standard addition as conventionally applied and in the modification intended here assumes a linear relationship between the meter reading and the concentration of the solution aspirated. Consequently

$$R_n = KC_n \tag{1}$$

is a condition that must be fulfilled.

For the sake of having a simple situation, the line obtained in a plot of R_n versus nS, the volumes of standard solution added should be straight ones. Then extrapolation towards the intercept with the nS-axis is readily possible and the intercept value can be used to compute the desired value C.

In order to investigate the situation the function $R_n = f(nS)$ must be established. Because of the linear relationship according to (1) it

will simplify the calculations when, as a first step, the question is reduced to the problem of expressing C_n as a function of the amount of standard solution added, that is, of nS. The formula for the solution concentration after the first step is

$$C_1 = \frac{(V-A)C + ST}{V-A+S} \tag{2}$$

Denoting the denominator in (2) as V_1, the concentration after two steps is given by

$$C_2 = \frac{(V_1-A)C_1 + ST}{V_1-A+S} \tag{3}$$

Substituting for V_1 and C_1 yields

$$C_2 = \frac{(V-2A+S)\,[(V-A)C + ST] + ST(V-A+S)}{(V-A+S)\,(V-2A+2S)} \tag{4}$$

Progressing in an analogous way the expressions for C_3 and C_4 are obtained

$$C_3 = \frac{(V-3A+2S)\{(V-2A+S)[(v-A)C+ST] + ST(V-A+S)\} + ST(V-A+S)(V-2A+2S)}{(V-A+S)(V-2A+2S)(V-3A+3S)} \tag{5}$$

$$C_4 = \frac{\begin{array}{l}(V-4A+3S)\,[(V-3A+2S)\{(V-2A+S)[(V-A)C+ST] + ST(V-A+S)\} + \\ + ST(V-A+S)(V-2A+2S)] + ST(V-A+S)(V-2A+2S)(V-3A+3S)\end{array}}{(V-A+S)(V-2A+2S)(V-3A+2S)(V-4A+4S)} \tag{6}$$

Equations (2) through (6) suffice to establish the pattern that would allow one to write down the equations for C_5, C_6, and so forth. The equations show that the formulations are too complex and difficult to

handle when trying to judge the practical usefulness of a situation.

As stated, any actual analytical use without extensive calculation would require a straight-line graph. Thus, the question arises whether there is a possible straight-line relation and if so under which conditions. Trials to develop a general equation ended in frustration and finally the following trick was applied. If a straight-line relationship exists, the differences between two consecutive points must be equal. Some differences were calculated to be as given in Equations (7), (8), and (9)

$$C_1-C = S\,\frac{T-C}{V-A+S} \tag{7}$$

$$C_2-C_1 = S(V-A)\,\frac{T-C}{(V-A+S)(V-2A+2S)} \tag{8}$$

$$C_3-C_2 = S(V-A)(V-2A+S)\,\frac{T-C}{(V-A+S)(V-2A+2S)(V-3A+3S)} \tag{9}$$

Setting the first two differences equal leads to

$$\frac{S(T-C)}{V-A+S} = S(V-A)\,\frac{T-C}{(V-A+S)(V-2A+2S)}$$

Cancelling the appropriate terms gives

$$1 = \frac{V-A}{V-2A-2S}$$

from which the condition for a straight-line plot derives as

$$A = 2S \tag{10}$$

The same result is obtained from equating the second pair although considerably more shuffling around is necessary. An easier way is to substitute the value of 2S for the A in Equations (8) and (9) and obtain verification of the straight-line relationship via the identity of the rearranged expressions.

Substitution of A according to (10) in (2), (4), and (5) yields

$$C_1 = C + S\,\frac{T-C}{V-S} \tag{11}$$

$$C_2 = C + 2S\,\frac{T-C}{V-S} \tag{12}$$

$$C_3 = C + 3S\,\frac{T-C}{V-S} \tag{13}$$

From the recognizable pattern the general equation can be readily established as

$$C_n = C + nS\,\frac{T-C}{V-S} \tag{14}$$

This yields a straight line when plotting C_n as a function of nS, the amount of standard added.

Combination of (1) and (14) gives the equation for the analytical plot

$$R_n = K\left(C + nS\,\frac{T-C}{V-S}\right) \tag{15}$$

The way of operation in practice is, of course, in the opposite direction. The plot is established from experimental data and the unknown C is obtained from it. The equation to compute the concentration of the sought-for substance in the sample can be derived as follows. The zero intercept (at the abscissa) is the value of nS when $R_n = 0$ and may be denoted as $(nS)_o$. These conditions lead to

$$K\left[C + (nS)_o \frac{T-C}{V-S}\right] = 0 \tag{16}$$

and after rearranging, the desired formula results as

$$C = \frac{(nS)_o\, T}{(nS)_o - V+S} \tag{17}$$

Practical Considerations

It follows from the derivations that during one run of a determination the amount of S has to remain constant; therefore, the scale reading can be expressed as a function of n, the number of additions insdead of nS, the added volumes. Then the scaling of the abscissa to the right of the origin is the same in every determination, involving simply the integers 1, 2, 3, ... The extrapolated zero intercept of course is not necessarily an integer as it indicates what fraction of an addition, so to speak, is equivalent to the amount of sought-for substance in the sample. When plotting meter reading versus n, the following expression is to be used

$$C = \frac{PST}{PS-V+S} \tag{18}$$

where P is the intercept at the X-axis when n is plotted on that axis. A reminder: For actual calculations it should be remembered that P (as would be $(nS)_o$) is a negative number!

From a practical point of view the following additional considerations are of value. Depending on the situation the most suitable concentration of the standard solution, T, will differ from case to case. Thus it would seem necessary to have a series of standard solutions at hand. It would be much simpler and is readily possible to operate with one standard only. This standard solution is quite concentrated and delivered from a microburet or dispensed with small volume pipets. Then, solvent, buffer, or whatever solutions need to be used are employed to make up the rest of the addition to exactly the amount of S. Under these conditions the concentration of standard in the replacing solution, that is, the T, is obtained from the volume, Q, and concentration, W, of the concentrated standard by

$$T = \frac{QW}{S} \tag{19}$$

Replacement of T in (18) leads then to the final equation covering all practical cases in the best manner

$$C = \frac{PQW}{PS-V+S} \tag{20}$$

where

C is the concentration of sought-for substance in the original sample and has the same units in which W is expressed,

V is the initial volume of sample solution,

S is the total volume that has been replaced in each step after 2S has been withdrawn for measurement (S may be a composite of Q plus solvent or buffer),

Q is the volume of standard added (if the standard is all that is added, Q=S),

W is the concentration of the sought-for substance in the standare solution, and

P is the zero intercept at the X-axis when meter readings (on Y-axis) are plotted versus the number of additions.

Experimental

Solutions of various metals were prepared in the usual way from Reagent Grade material; distilled, deionized water was employed throughout. The experimental runs were performed on a variety of instruments including a Perkin Elmer Model 403 atomic absorption unit and a Coleman Model 21 flame emission spectrophotometer.

Procedural Details

Instead of giving a general procedure, it seems more suited in the present case to give a detailed description of exactly how one particular analysis was performed. The example involves the determination of copper using a Perkin Elmer Model 403.

1) The atomic absorption instrument was adjusted to suitable conditions and the zero set with water being aspirated into the flame.

2) 12.00 ml of the sample solution was placed in a dry beaker.

3) Solution was withdrawn with a 4-ml pipet, and 4.00 ml placed into a dry, disposable cup, and from there aspirated into the flame. The reading (0.063) was taken and plotted as R_o in Figure 10. Care was exercised not to lose any solution during and after the transfer.

4) From a finely graduated buret, 0.20 ml, 63.5 ppm standard of $CuSO_4$, was added to the remaining sample solution and then 1.80 ml of water from another buret to yield a total of 2.00 (=S) ml being added, exactly half of what had been withdrawn. The solution was well mixed.

5) The 4-ml pipet used in step (3) was rinsed by twice drawing up solution and emptying back into the beaker. Then another 4-ml volume was withdrawn, placed in a new cup, and the liquid was aspirated into the flame; the reading 0.101 was taken and plotted as $R_1(0.101)$.

6) Steps (4) and (5) were then repeated several more times and the respective readings plotted as $R_2(0.134)$, $R_3(0.177)$, and $R_4(0.212)$.

7) The best line was drawn through the points and extrapolated to the intersect.

8) The value of the intersect P = -1.70 was taken and in connection with other data required substitution into Equation (20)

$$C = \frac{-1.70 \times 63.5 \times 0.20}{-1.70 \times 2 - 12 + 2}$$

given the value for the unknown to be C = 1.61 ppm; the theoretical value was 1.59 ppm.

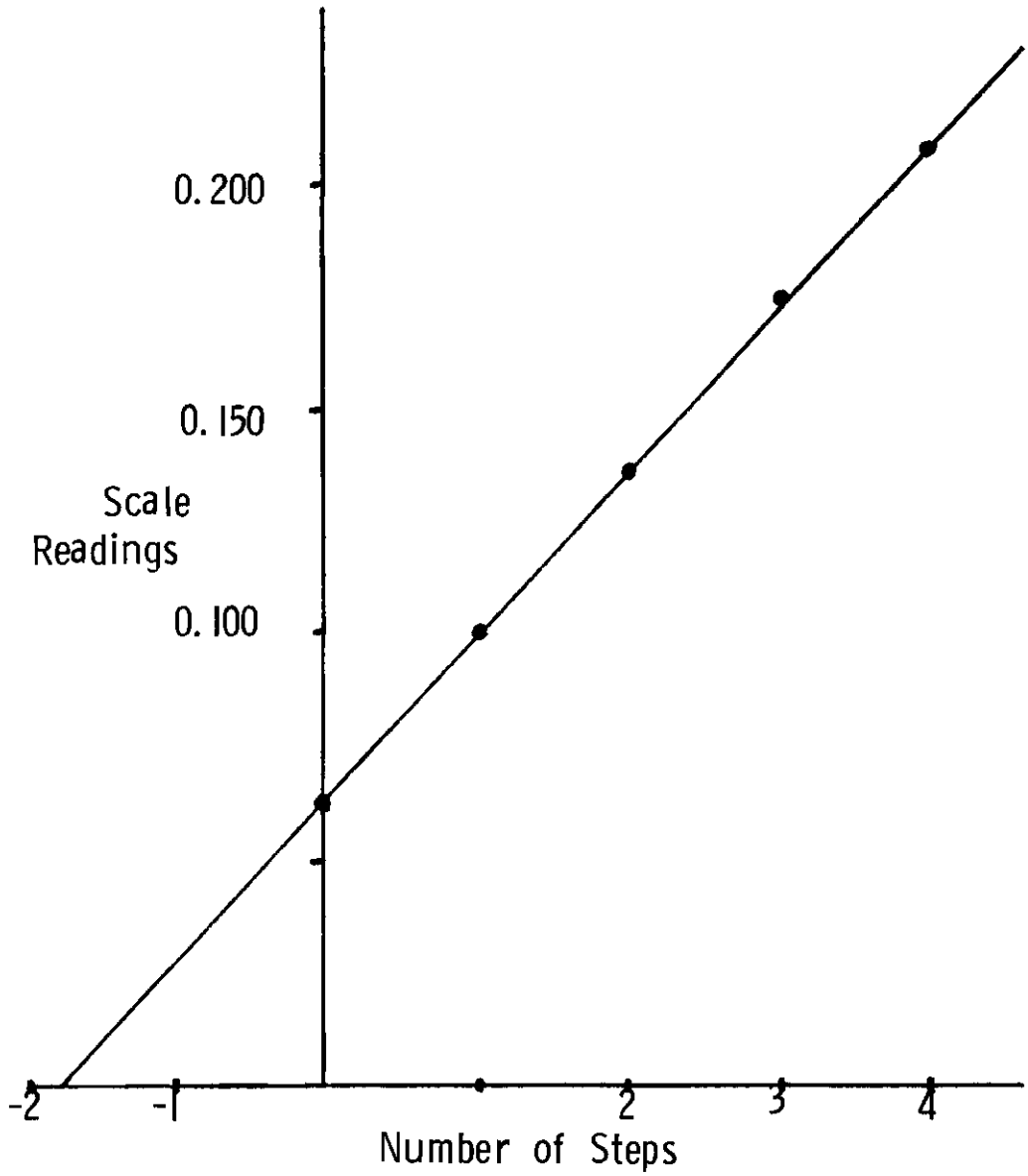

Figure 10

Graph of Experimental Data of Analysis
Described in "Procedural Details"

Results and Discussions

When using a common pipet, calibrated "TD" (to deliver), somewhat more than the intended volume is withdrawn at least in the first step. This excess, consisting of the drop filling the tip and the amount required to wet the walls of the pipet, is quite small and as actual determinations have shown, negligible. In addition, an assumed example was treated mathematically and the first withdrawal taken by +10% in error (V=10, 2S=2.20 instead of 2.00). This deviation is, of course, far greater than any that may be encountered in reality. The result was C = 4.91 instead of the expected 5.00. Thus, the influence of actually occurring errors must indeed be very small. However, care should be taken that such errors are not introduced in every other step after the first one. Therefore, the rinsing of the pipet with solution as described in step (5) of the Procedural Details is enacted. In order to avoid loss of a drop from the pipet tip, it is recommended to store the pipet horizontally on a rack. The possibility may be considered to employ unwettable plastic pipets or pipets calibrated "TC" (to contain), and to siliconate them so that the walls are rendered unwettable.

Several practical considerations arise in connection with the various steps in the Procedure. What should be the size of the volume withdrawn, that is, of 2S? Generally this volume is not too critical as long as it is big enough to provide sufficient liquid so that a reliable reading can be made. In cases where strong matrix effects prevail, the withdrawal should be as small as possible in order to avoid extensive reduction of the originally present amount of matrix.

The question of how much standard is to be added is closely related

to the scaling employed in the graph. On the abscissa (X-axis) the scaling is to be selected so that the reading of the intercept can be made with no less reliability than is that of the measurements of the volumes. The scaling on the ordinate (Y-axis) should allow reflection of the precision of the instrument reading. With such scalings a slope of unity should approach optimal conditions. A quite comfortable situation is created when the reading after the first addition is about 50 to 100% higher than that obtained with the original solution. To achieve such a reading it is necessary to know from previous analyses or from special tests approximately what reading a certain volume of standard solution will give in a certain column of sample solution. The addition effected is then selected accordingly.

Scaling of the graph is of no moment when the computer program is used. But even with the program employed, it is recommended to plot a graph in order to detect any point that is obviously wrong because of misreading the scale or a similar mishap.

It should be emphasized that any loss of solution, any error in the volume added or withdrawn will make the step where it occurred, and of course all subsequent steps, useless.

Some representative results are compiled in Table 9 which allow the estimation of the reliability attainable at the levels employed. It should be kept in mind that with less sample solution a greater number of points was obtained than would have been possible with the common mode of operation.

Table 9. Representative Results from Determinations Using the New Method

V,ml	S,ml	Q,ml	W,ppm	R Values	C,ppm taken	C,ppm found
10.0	1.00	1.00	10.0	10.5 ; 21.0 ; 31.0 41.0 ; 52.0	1.00	1.02
5.00	0.50	0.50	10.0	6.0 ; 11.8 ; 17.2 23.2 ; 29.0	1.00	1.03
5.00	0.50	0.50	10.0	5.0 ; 9.5 ; 14.0 19.0 ; 24.0	1.00	1.01
5.00	0.50	0.50	20.0	2.5 ; 9.0 ; 16.0 22.0 ; 26.5	1.00	1.03
10.00	1.00	1.00	6.35	0.015 ; 0.031; 0.044 0.060 ; 0.076; 0.091	0.64	0.66
12.00	2.00	2.00	6.35	0.063 ; 0.101; 0.134 0.177 ; 0.215	1.59	1.59
5.00	0.50	0.50	6.35	0.015 ; 0.031; 0.046 0.062 ; 0.078; 0.091	0.64	0.64_5
10.00	1.00	0.10	63.54	0.015 ; 0.031; 0.045 0.061 ; 0.076; 0.092	0.64	0.66
10.00	2.00	1.00	0.63	0.002 ; 0.005; 0.007 0.0085	0.063	0.056
10.00	2.00	1.00	0.63	0.0025; 0.005; 0.0074 0.0098	0.063	0.065

NOTE: The first four examples involve flame emission determination of potassium; the last six examples involve atomic absorption determination of copper.

The computer program which follows is written in BASIC RT and was used on a PDP-8e. The computer is provided with initial volume, volume of standard added, concentration of standard, total replacement volume, and the scale readings (Y-axis values). The number of additions (X-axis values) is established automatically. A least square approximation is employed to give the value of the intercept and that value is used in connection with Equation (20) to give the concentration of the sought-for substance in the initial solution. The number of steps, N, the value of the intercept, P, and the unknown concentration, C, are printed out. An actual "RUN" is shown as insert, with the data of the determination described in the Procedural Details.

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10 REM PROGRAM FOR  STANDARD ADDITION METHOD WITH SAMPLE CONSUMPTION
20 REM PLOT OF SCALE READINGS VERSUS NUMBER OF ADDITIONS.
25 REM FEED TO COMPUTER ONLY SCALE READINGS AND ONLY ONE PER LINE.
30 REM WHEN ALL DATA  ARE IN PRINT 999 AND PROGRAM WILL START.
40 V=0\S=0\Q=0\W=0\N=0\F=0\G=0\H=0\L=0\P=0\C=0
45 A=2
48 FOR X=0 TO 1 STEP .05\PLOT X,0\NEXT X
49 FOR X=0 TO 1 STEP .1\PLOT .4,X\NEXT X
50 PRINT "INITIAL VOLUME V = ";\INPUT V
60 PRINT "ML STANDARD Q = ";\INPUT Q
70 PRINT "CONC. STANDARD W = ";\INPUT W
80 PRINT "TOTAL ML REPLACED S = ";\INPUT S\PRINT
100 PRINT "SCALE READINGS"
110 INPUT Y(N)\IF Y(N)=999 THEN 150
115 FOR X=1 TO 5\PLOT N/10+.4,A*Y(N)\NEXT X
120 F=F+N\G=G+N↑2\H=H+Y(N)\L=L+N*Y(N)\N=N+1\ THEN 110
150 PRINT "N="N
280 P=-(F*L-G*H)/(F*H-N*L)\PRINT "P = "P
300 C=(P*Q*W)/(P*S-V+S)\PRINT "C = "C
310 FOR I=P TO 10+P STEP .1
320 T(I)=((F*H-N*L)/(F↑2-N*G))*I+(F*L-G*H)/(F↑2-N*G)
330 PLOT I/10+.4,A*T(I)\DELAY\NEXT I
```

```
RUN
INITIAL VOLUME V = ?12
ML STANDARD Q  = ?.2
CONC. STANDARD W = ?63.5
TOTAL ML REPLACED S = ?2

SCALE READINGS
?.063
?.101
?.134
?.177
?.212
?999
N= 5
P = -1.673797
C =  1.592588

READY .
```

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